ON ROUS, LEUCOTIC AND ALLIED TUMOURS IN THE FOWL

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BY THE SAME AUTHOR

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PERNICIOUS ANAEMIA, LEUCAEMIA, AND APLASTIC ANAEMIA. An Investigation from the Comparative Pathology and Embryological Point of View.

ON ROUS, LEUCOTIC & ALLIED TUMOURS IN THE FOWL

A Study in Malignancy

BY

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WITH 21 ILLUSTRATIONS (1 COLOURED)

THE MACMILLAN COMPANY
NEW YORK
1928

PRINTED IN GREAT BRITAIN

PRINTED IN GREAT BRITAIN BY ROBERT MACLEHOSE AND CO. LTD.
THE UNIVERSITY PRESS, GLASGOW.

INTRODUCTION

DURING the period when leucosis [1] of fowls was under investigation and subsequently, several sporadic cases of sarcomatous tumours of the fowl were met with. These resembled closely the classical Rous sarcoma No. 1. A study of them forms the basis of the present work.

One could not, however, work simultaneously on leucosis of fowls with tumour formation and on these tumours without being struck with an essential resemblance between them. The endeavour will be made to show that this consists in their both being tumours of the haematopoietic tissues brought about by essentially similar causes.

Some cases of the Rous type of tumour were encountered which showed the presence of melanin in large quantities. They seemed to afford an opportunity for the study, amongst other things, of the origin of melanin and, accordingly, they are discussed in this connection.

Although, in leucosis of fowls, tumours of a 'lymphoid' nature are common, the lymphatic system of the fowl is characterised by the absence of lymphatic glands and some other lymphatic structures. This fact, together with the failure to establish the existence in the fowl of a leucosis of true lymphatic type, renders necessary a discussion of some points in regard to the lymphatic system of the fowl. This involves a consideration of the histogenesis of the lymphocyte and a broadening out of the inquiry to deal with allied problems in mammals.

As considerable confusion exists in most discussions of haematological subjects directly attributable in the main

[1] *Vide* McGowan, *Pernicious Anaemia, Leucaemia and Aplastic Anaemia*; 1926. London, H. K. Lewis & Co.

to the confused use of terms, a short chapter will be introduced at the outset dealing with the genealogy of blood cells under an attempted uniformity of designation. The origins and names so apportioned will be upheld as far as possible in the discussion. If this serves no other purpose, it will, at least, tend to make an avowedly difficult subject more easy to treat.

The classical Rous tumour No. 1 has also been made the subject of investigation. This work, although undertaken subsequent to the observations on the sporadic tumours, is dealt with first. It will serve to supply a standard with which the other tumours can later be compared. The particular strain of Rous tumour No. 1 used, was supplied me through the kindness of Dr. Archibald Leitch, Cancer Hospital Research Institute, London.

It has been found impossible to acknowledge the source of all the information made use of in the text. Special mention, however, may be made here of the following: Dawson's *Melanomata ;* Jolly's *Traité technique d'hématologie;* Naegeli's *Blutkrankheiten und Blutdiagnostik ;* Maximow's various papers ; and Sharpey-Schafer's *Endocrine organs.*

I had asked my friend Dr. James Dawson, the author of *Melanomata* already referred to, to help me with the interpretation of some histological appearances arising in connection with this work. There is no one who more deservedly had the right to be considered as one of the greatest authorities in this sphere. With the entire unselfishness and wholehearted interest in probing for the truth, which characterised him, he readily agreed. His long illness, and finally his death, prevented his accomplishing it.

I am indebted to Miss Mursell, B.Sc., for the coloured drawing, for the provision of material, and for help in various ways ; to Mr. Wm. Ramsay, Dyce, for preparing the microphotographs, and to the Committee and Director of the Rowett Institute for the facilities afforded for carrying out the work.

June, 1927.

CONTENTS

ON ROUS, LEUCOTIC AND ALLIED TUMOURS IN THE FOWL

I

BLOOD CELL ORIGINS AND NOMENCLATURE

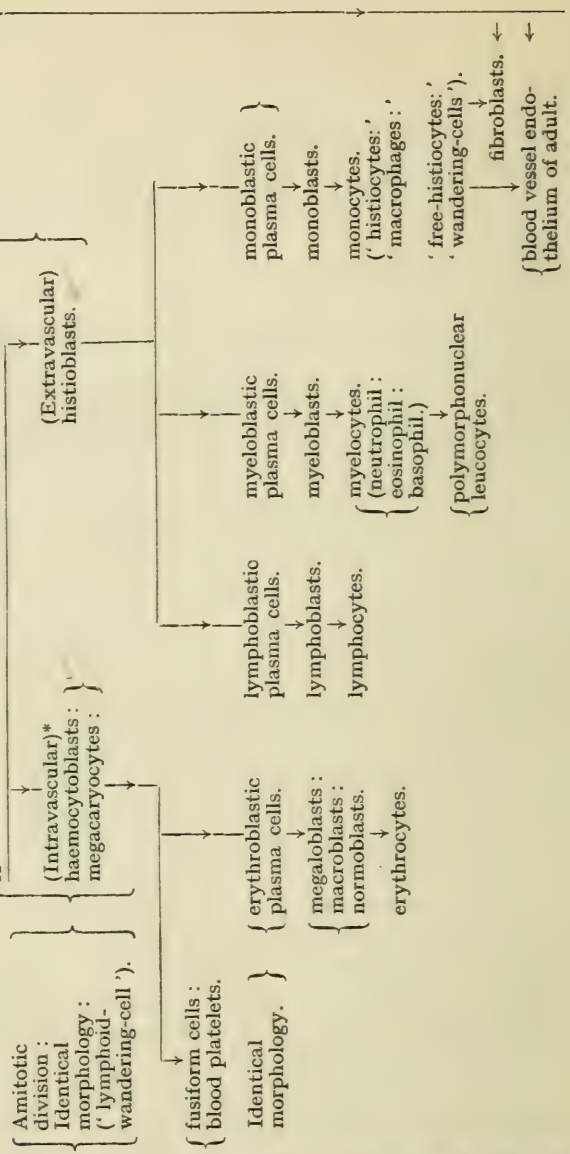
It seems advisable, before proceeding to discuss the main theme, which has largely to do with haematopoietic tissues, to attempt to outline a scheme of the origin of blood cells and to specify briefly the signification of certain terms which will of necessity be used frequently. This it is hoped will tend to clarity and ease of exposition.

The easiest and shortest way of carrying out this intention appears to be by means of a suitable diagram. The scheme, to be outlined in Fig. 1, has been arrived at in the main from a consideration of Maximow's (1) and Aschoff's (2) articles and from personal observations. With regard to the latter, these have been discussed in relation to the erythrocytic side of haematopoietic function elsewhere (3). *Mutatis mutandis* the statements, made there in this regard, are in the main applicable to the myelocytic, lymphocytic and monocytic activities. Hence, no elaborate discussion is necessary here except what may be with advantage given as explanatory of the diagram on page 2.

From the diagram it will be seen that, according to the present conception, the mesenchyme cell gives rise to the haemohistioblast from which two main streams diverge an intra- and an extra-vascular one. The intravascular one, A, gives rise to those cells which of necessity perform their functions inside vessels and cannot be conceived as having any possible function to perform outside. Such cells are the

FIGURE 1

Mesenchyme, including reticulo-endothelial system in the narrow sense ('resting-wandering-cell,' 'fixed-histiocyte').†



* An intravascular monocytic lineage derived from such cells as the embryonic endothelium and the Kupffer cells of the adult liver should have been shewn. Omitted for sake of clearness.

† Ferrata's 'Hæmohistioblast.'

erythrocytes, the fusiform cells, and blood platelets. The extravascular one, B, produces cells extravascularly which perform most of their functions extravascularly. When they appear in the blood vessels, as they do in physiological and pathological conditions, it would seem that they are using them mainly as ready channels to get from one extravascular position of the body to another to carry out, there, their essential extravascular function. The term haemohistioblast would appear to be a convenient designation for these two types of cells before they have definitely declared themselves.

It will be seen that the haemocytoblast and the histioblast, together with the haemohistioblast, are of identical morphology, that of the lymphoid-wandering-cell of Maximow. Further, amitotic division is predicated for the cells at this stage of development. The reasons for assuming this are discussed elsewhere (McGowan, *loc. cit.*). At other stages, division would seem to be of the ordinary mitotic type. Plasma cells would appear to be an essential intermediate stage between the haemocytoblasts and histioblasts on the one hand and the precursors of the various -cytes on the other. They are identical morphologically with the exception that the erythroblastic plasma cell has haemoglobin in its cytoplasm. The term plasma cell is therefore rather a morphological than a physiological one.

Most of the lineages, especially when they get beyond a certain developmental stage, are irreversible. This seems to be the case for the erythroblastic one when it reaches the stage of the erythroblast, and similarly for the lymphoblastic and myeloblastic. It is possible, however, that, with regard to the monocytic lineage, a condition of greater or less reversibility may be present. At both ends of the lineage phagocytosis is a marked characteristic, one group being sessile phagocytes, the other actively motile ones ; at both ends of the lineage, too, further developmental potencies are relinquished by both groups of cells becoming blood-vessel-endothelium of the adult or fibroblasts.

Many terms have been used to designate the cells of this

lineage. It would seem that ' fixed-histiocyte ' at one end and ' monocyte ' or ' free-histiocyte ' at the other are as good as any (' monocyte ' being used for the blood circulating cell, ' free-histiocyte ' for the cell in the tissues). These will accordingly be used for the cells in question in the subsequent discussion.

II

OBSERVATIONS ON ROUS TUMOUR No. 1

A SAMPLE of Rous tumour No. 1 was obtained from Dr. Archibald Leitch. This was inoculated into a series of fowls for the purpose of eliciting the nature of the local and general spread, the changes in the circulating blood, and to provide material for the experiments to be described in Chapter III.

In the first instance, two white leghorn cocks, 8 months old, cocks 84 and 86, were inoculated subcutaneously over the right breast with the tumour as received. The salient features in the two cases were as follows :

Case 1.—Cock 86. On 11th December, 1926, when inoculated, weighed 1900 grammes : on 6th January, 1927, when killed *in extremis*, the weight was 1730 grammes. The following blood phenomena were observed :

	Polymorphs.[1]	Monocytes.	Lymphocytes.	Masts.
11th Dec., 1926	35.4	8.5	55.3	0.8
14th ,,	46.0	11.0	42.0	1.0
21st ,,	40.0	23.8	36.0	0.2
22nd ,,	46.4	23.9	27.8	1.9

On 22nd December, 1926, red blood cells were 3,700,000 per c.mm. ; white blood cells were 66,000 per c.mm. A few normoblasts seen.

On 6th January, 1927, its comb was dark in colour, the bird was droopy and cold, and blood was not easily obtained for examination. There was a large tumour on the posterior

[1] The two varieties are enumerated together.

part of the right breast, partly covered by a dry scab. It was killed. *Post mortem*, there was great emaciation : on posterior part right breast and spreading over abdomen a tumour of the size of a Jaffa orange : superficial part of the tumour is yellow and necrosed : deeper part near the muscle is pure white. Invasion of the underlying muscle is taking place by tongue-like processes or round nodular lumps, some of these at quite a distance from the body of the tumour : no tumours seen elsewhere in the muscles. There was no direct spread from the tumour to the body cavities.

The abdomen was full of blood-stained fluid derived from the liver : a large part of the liver was necrosed and contained tumour masses and haemorrhages : the other part was more or less normal but contained very numerous discrete, circumscribed, round nodules of various sizes, some of them very small. They were pure white, solid and hard, and some of them projected above the surface of the liver. There was no sign of necrosis in these tumours, which were, however, relatively small. They appeared to be growing in the periportal spaces.

The spleen was normal. There were discrete little white tumours in the left kidney—none in the right ; several nodules of the same type occurred in the auricles and ventricles of the heart, and the lungs were full of similar discrete white nodules.

The medulla of the femur and tibia were distended with pale grayish marrow : the metatarsal bone showed fat only.

In the internal organs the tumour nodules remained confined to the organ in which they arose and there was no tendency to indiscriminate infiltration to adjacent organs. The large tumour masses were made up from the coalescence of a large number of small independent foci, each of which spread peripherally.

Organs and tissues not referred to specifically appeared to be normal. The thymus was not enlarged.

Case 2.—Cock 84. On 11th December, 1926, its weight was 2140 grammes, while on 6th January, 1927, this had fallen to 1730 grammes.

The following blood phenomena were observed :

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
11th Dec., 1926	39.8	13.1	45.6	1.5
14th ,,	49.7	17.0	32.4	0.9
21st ,,	30.0	39.5	30.0	0.5
22nd ,,	20.0	38.0	40.0	2.0

On 22nd December, 1926, red blood cells were 3,550,000 per c.mm. ; white blood cells, 18,000 per c.mm. No normoblasts found.

On 10th January, 1927, the haemoglobin, which previously had been about 70 per cent., had dropped to 50 per cent. : the comb was pale : and the blood film showed a marked normoblastic reaction. It was killed. *Post mortem*, there was found extreme emaciation. A tumour of the size of a Jaffa orange was present over the posterior part of the right breast, and extended from there over the abdomen. The tumour had much the same appearance as in cock 86, but the little round white nodules in the substance of the muscle away from the body of the tumour were much more marked. The surface of the tumour towards the muscle, when pulled off the muscle, had the mammillated appearance of the surface of a bunch of grapes. There was no invasion of the body cavity direct from the tumour mass. The lung showed a few white nodules but less than in cock 86, the central parts of the lungs being chiefly affected ; there was no tumour in the heart : the thymus was small : the liver was dark brown, showed no tumour masses, but close inspection showed minute white streaks running all through and between the lobules : the spleen was slightly enlarged as were also the kidneys, but neither showed tumour masses : the medulla of the femur and the tibia were filled with light yellowish pink marrow without any cancellous trabeculae : the metatarsal showed fat only.

The histological appearances found were briefly as follows : In both cases the necrotic portion showed cell debris—the cells present in such positions being few in number and often

of the large free-histiocytic type (macrophages). The necrotic portion was often delimited by a dense layer of nuclei of dead cells, apparently of the polymorph type. With regard to the ' healthy ' white tumour, this consisted of an interlacing network of spindle and stellate cells of open character with a tendency to mucoid degeneration. At the advancing margin of the body of the tumour the cells were of two types, first, the free-histiocyte type, of all sizes, some enormous and with a marked tendency to mitosis, grading down to cells practically indistinguishable from lymphocytes, and, secondly, typical histioblasts of angular shape irregularity in size, and deep basophile cytoplasm.

Mitotic figures were never observed in the stellate or spindle cells : the increase in size of the tumour seemed to take place by mitotic divisions in the free-histiocytes or by amitotic division in the histioblasts where they occurred.

The perivascular origin of the tumour would appear to be reflected in the whorled arrangement of the tumour tissue round the vessels in the body of the tumour. The nodules away from the body of the tumour showed a central artery surrounded by a mass of cells, consisting of histioblasts, free-histiocytes of all sizes and, in the larger nodules, stellate and spindle-shaped tumour cells.

This description holds for the subcutaneous mass. The appearances were much the same in the tumours in the various organs. A marked exception, however, was found in the tumours in the liver of cock 86. It should be mentioned that in this organ, as judged of by the very small foci, the tumour occurred in the periportal spaces. In the larger nodules in this situation, in addition to the histological findings already described, there were present large numbers of cells with eosinophil granules. Such cells were not found in the ' healthy ' tumour in other situations. They occurred sometimes isolated, sometimes in large clumps. Some of them had a polymorph nucleus, in others this could not be detected and they appeared to be myelocytes. In cock 84, on the other hand, where there were no macroscopic

tumours, every periportal space showed marked hyperplasia. The cells present were histioblasts and myelocytes in many of the spaces, whilst, in others, true tumour cells of the stellate and spindle type were found mixed up with these.

In the lung, the tumour nodules appeared to arise in the interlobular tissue and then to spread radially into the lobules.

Haemorrhages were present in both cases in the substance of the tumour : they were large, however, and their exact genesis could not be determined. Their occurrence and formation will be discussed more fully in regard to later cases.

In both cases there was marked activity of the bone marrow, which was preponderatingly myelocytic in type : no tumour tissue was observed in it.

On 6th January, 1927, cocks 81, 82, 83, 85, 87 and 88 were inoculated subcutaneously over the posterior part of the left breast with a small piece of tumour from cock 86 : while on 11th January, 1927, cocks 89, 90, 17, 18, 19, 20 and 21 were similarly inoculated with the tumour from cock 84. These were all white leghorn cocks of the same batch as cocks 84 and 86 already dealt with. At the outset, it may be stated that the results of these two series of inoculations were quite different from those in cocks 84 and 86. In many of the birds, the tumour development was of the slightest even after two months, and the large majority did not die from the tumour, but were killed for some special purpose. Nevertheless, the mixed dried powder from the tumours of cocks 84 and 86, when inoculated in May, 1927, in small quantity into a 12 weeks old barred Plymouth rock chicken, killed it in three weeks with a massive tumour locally and complete solidification of the lungs.

The history of these thirteen inoculated cocks will now be briefly detailed. Some of them served for the trypan blue experiments to be described in Chapter III, so that only what concerns the subject matter of this Chapter will be discussed here.

Case 3.—Cock 85, inoculated 6th January, 1927: on 24th January, 1927, it showed no tumour and was inoculated with trypan blue: blood films showed as follows:

	Polymorphs.[1]	Monocytes.	Lymphocytes.	Masts.
6th Jan., 1927	18	14	16	4
13th ,,	9	18	67	6
18th ,,	35	9	54	2

Died on 26th January, 1927, as the result of the trypan blue inoculation. No tumour anywhere.

Case 4.—Cock 17, inoculated 11th January, 1927: on 25th January, 1927, examined—no tumour palpable and inoculated with trypan blue: blood films showed as follows:

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
17th Dec., 1926	13	18	65	4
11th Jan., 1927	30	18	50	2
18th ,,	32	13	53	2
24th ,,	20	20	56	4

Cock very ill on 26th January, 1927, as result of trypan blue inoculation. Killed—no tumour found anywhere.

Case 5.—Cock 18, inoculated 11th January, 1927: on 26th January, 1927, no tumour palpable. Inoculation with trypan blue commenced: blood films showed as follows:

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
7th Dec., 1926	39	5	54	2
14th ,,	43	6	50	1
11th Jan., 1927	38	7	51	4
18th ,,	37	8	52	3
24th ,,	35	16	46	3

[1] The two types of polymorphs are taken together, while the percentages are given in round figures.

On 1st February, 1927, very ill as result of trypan blue inoculation : killed. Showed a small tumour at site of inoculation of the size of a marble : tumour not infiltrating, quite freely movable : central part of the tumour necrotic, peripheral ' healthy.'

Case 6.—Cock 81, inoculated on 6th January, 1927 : on 24th January, 1927, showed a good sized tumour at the site of inoculation : on 26th January, 1927, inoculation with trypan blue commenced : blood films showed as follows :

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
6th Jan., 1927	22	17	60	1
13th ,,	8	10	80	2
18th ,,	10	11	78	1
24th ,,	25	30	42	3
26th ,,	39	44	14	3

On 30th January, 1927, very ill as result of trypan blue inoculation. Killed : showed a large tumour infiltrating the posterior part of the breast and the abdominal wall : the superficial layer of the tumour was necrotic : no invasion of the abdonimal cavity. There was a small nodule of the tumour in the left kidney : no tumour nodules elsewhere.

Case 7.—Cock 87, inoculated 6th January, 1927 : on 24th January, 1927, noted as having a large tumour at site of inoculation : on 1st February, 1927, tumour size of a hen's egg. Inoculation with trypan blue commenced : blood films showed as follows :

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
13th Jan., 1927	27	6	65	2
18th ,,	30	12	56	2
24th ,,	20	37	40	3
31st ,,	25	30	43	2
1st Feb., 1927	17	22	60	1

Cock killed on 8th February, 1927 : tumour in this case round, circumscribed, non-infiltrating, size of hen's egg,

central part necrotic, peripheral part ' healthy '—no tumour elsewhere.

Case 8.—Cock 21, inoculated 11th January, 1927 : on 24th January, 1927, noted as having a ' fair ' tumour : on 31st January, 1927, tumour size of a hen's egg : inoculation with trypan blue commenced : blood films showed as follows :

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
7th Dec., 1926	15	20	62	3
14th ,,	12	13	73	2
11th Jan., 1927	18	13	66	3
18th ,,	16	10	71	3
31st ,,	19	25	55	1
1st Feb., 1927	5	25	67	3

On 7th February, 1927, cock, as the result of the trypan blue inoculation, was very dull. Killed. The local tumour was the size of a hen's egg, without infiltration, necrosed in the centre, ' healthy ' at the periphery—no tumour elsewhere in the body.

Case 9.—Cock 20, inoculated 11th January, 1927 : tumour on 31st January, 1927, was size of a large bean : on 7th February, 1927, it was the size of a hen's egg : on 8th February, 1927, inoculation of trypan blue commenced : the following were the film results :

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
7th Dec., 1926	29	14	66	1
14th ,,	8	16	75	1
11th Jan., 1927	7	13	77	3
18th ,,	14	14	58	4
24th ,,	19	20	60	1
31st ,,	12	27	60	1
7th Feb., 1927	27	32	40	1
8th ,,	16	22	60	1

On 7th February, 1927, red blood corpuscles = 3,300,000 per c.mm. and white blood corpuscles = 20,000 per c.mm.

On 8th February, 1927, red blood corpuscles = 2,750,000 per c.mm. and white blood corpuscles = 25,000 per c.mm.

Cock 20 died on 11th February, 1927 : tumour size of hen's egg : rounded : centre necrotic : surface next the muscle invading it with round nodules in advance of the main mass ; about half a dozen round sharply circumscribed tumour nodules in the liver from size of a pea downwards, some of them contained blood cyst in the centre. The lungs are both solid with coalescing round small nodules of the tumour : some of these have blood cysts in centre : the heart shows two small nodules in the ventricle.

Case 10.—Cock 90, inoculated 11th January, 1927 : on 31st January, 1927, tumour recorded as size of a walnut : on 7th February, 1927, tumour size of a hen's egg : on 8th February, 1927, trypan blue inoculations commenced : blood films gave the following results :

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
11th Jan., 1927	31	13	54	2
18th ,,	26	20	51	3
24th ,,	46	11	41	2
31st ,,	39	30	30	1
7th Feb., 1927	45	11	41	3
8th ,,	47	21	31	1

On 7th February, 1927, red blood corpuscles were 3,800,000 per c.mm. and white blood corpuscles were 47,500 per c.mm.

On 8th February, 1927, white blood corpuscles were 30,000 per c.mm.

Cock killed on 14th February, 1927. Tumour size of a hen's egg—round, non-infiltrating, necrotic in centre. No tumour elsewhere.

Case 11.—Cock 83, inoculated 6th January, 1927 : on 24th January, 1927, tumour recorded as size of walnut : on 7th February, 1927, same size, and was round and freely movable : on 9th February, 1927, cock so well that it had been fighting : tumour almost the size of a walnut round and freely movable : blood films showed following results :

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
6th Jan., 1927	20	8	70	2
13th ,,	35	9	54	2
24th ,,	41	6	51	2
31st ,,	12	24	61	3
7th Feb., 1927	50	8	39	3
9th ,,	34	28	34	4

On 7th February, 1927, red blood corpuscles were 3,000,000 per c.mm. and white blood corpuscles were 52,500 per c.mm. ; and on 9th February, 1927, red blood corpuscles were 3,450,000 per c.mm. and white blood corpuscles were 42,000 per c.mm.

Cock killed on 9th February, 1927. Tumour round, freely movable, no infiltration, central part necrosed : no tumours elsewhere.

Case 12.—Cock 19, inoculated 11th January, 1927 : on 31st January, 1927, tumour size of a walnut : on 7th February, 1927, tumour same size and pedunculated : blood films gave the following results :

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
14th Dec., 1926	26	15	55	4
11th Jan., 1927	15	12	71	2
18th ,,	45	15	38	2
24th ,,	62	7	30	1
31st ,,	53	22	22	1
7th Feb., 1927	54	13	32	1
9th ,,	53	31	15	1

On 9th February, 1927, red blood corpuscles were 3,000,000 per c.mm. ; white blood corpuscles were 62,500 per c.mm.

Cock killed 9th February, 1927, pedunculated tumour size of a walnut, freely movable, no infiltration : central part necrosed : no tumour elsewhere.

Case 13.—Cock 82, inoculated 6th January, 1927 : on 24th January, 1927, tumour was size of hazel nut : on 7th

February, 1927, it could not be palpated. Blood films gave the following results.:

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
6th Jan., 1927	24	15	58	3
13th ,,	31	17	49	3
18th ,,	17	17	63	3
21st ,,	23	23	52	2
24th ,,	20	28	49	3
7th Feb., 1927	27	16	54	3
10th ,,	35	19	42	4
15th ,,	34	19	44	3
21st ,,	22	20	55	3

On 10th February, 1927, red blood corpuscles were 4,000,000 per c.mm. and white blood corpuscles were 45,000 per c.mm.

On 15th February, 1927, red blood corpuscles were 3,200,000 per c.mm. and white blood corpuscles were 45,000 per c.mm.

Case 14.—Cock 88, inoculated on 6th January, 1927 : on 31st January, 1927, the tumour was pedunculated and the size of a walnut : on 7th February, 1927, the tumour was noted to be larger than a hen's egg with edges sloping into the surrounding tissues. The comb was slightly pale : haemoglobin was 35 per cent.

Red blood cells were 1,650,000 per c.mm. and white blood cells were 75,000 per c.mm. On 9th February, 1927, haemoglobin was 35 per cent. : on 10th February, 1927, haemoglobin was 35 per cent. : on 14th February, 1927, cock died. Blood films gave the following results :

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
6th Jan., 1927	63	22	14	1
13th ,,	53	21	25	1
18th ,,	47	21	29	3
31st ,,	50	18	30	2
24th ,,	47	30	19	4
7th Feb., 1927	85	13	1	1
9th ,,	62	27	8	3
11th ,,	64	27	6	3

From 7th February, 1927, a considerable number of erythroblasts appeared in the blood. *Post mortem* examination showed locally a roundish freely movable tumour, not infiltrating, with a necrotic centre. There were large blood clots in peritoneal cavity: these originated from the liver, which was much enlarged. It showed large, round, circumscribed, white tumour masses; similar tumour masses, with several round blood cysts inside them; large blood cysts with a thin strip of tumour tissue round them; huge haematomas, and tumour masses with necrotic areas inside them.

The lungs showed the characteristic round tumour masses becoming confluent. Some of these showed necrosis in their centres, others again, blood cysts. No tumour masses elsewhere.

Microscopically the liver showed: (1) Typical tumour tissue in the periportal spaces; (2) Histioblastic tissue with myelocyte formation; (3) Mixtures of (1) and (2). Practically every periportal space is affected.

The blood cysts in the interior of the tumour masses appear to be due to a weakening [1] and then disappearance of the blood vessel wall, round which the tumour develops originally. The walls of the blood cysts are formed by 'healthy' or necrosing tumour tissue. As the tissue necroses more and more, the blood cyst becomes larger and larger, until, finally, it is surrounded by a thin band of tumour tissue which may eventually rupture.

The tumour nodules increase in size at their periphery by means of small round free-histiocytes, which divide mitotically. These change into stellate branching and anastomosing cells which do not divide. The tumour tissue penetrates between the liver columns and gradually destroys them.

The tumour in the lung shows well the invasion of the lobules from the perilobular tissue.

[1] The fact of a small artery being in the centre of a nodule does not mean an increased blood supply to the tissue surrounding it. This tissue would be as liable to necrosis as the central portions of nodules without an artery where indeed necrosis is frequent.

Case 15.—Cock 89, inoculated 11th January, 1927: on 31st January, 1927, tumour size of hazel nut: on 7th February, 1927, tumour disappeared: blood films gave the following results:

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
11th Jan., 1927	37	12	49	2
18th ,,	30	17	51	2
24th ,,	30	16	52	2
31st ,,	20	25	51	4
7th Feb., 1927	41	10	46	3
15th ,,	27	12	59	2
16th ,,	33	11	53	3

On 17th February, 1927, red blood corpuscles were 3,650,000 per c.mm. and white blood corpuscles were 25,000 per c.mm.

Cock was killed: all organs healthy: no local tumour.

For comparison with the blood figures given above, those of a cock—cock 95—of the same batch which had not been inoculated will now be given:

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
10th Feb., 1927	19	22	56	3
15th ,,	30	20	48	2

On 10th February, 1927, red blood corpuscles were 3,200,000 per c.mm. and white blood corpuscles were 25,000 per c.mm.

On 15th February, 1927, red blood corpuscles were 3,650,000 per c.mm. and white blood corpuscles were 20,000 per c.mm.

Ellermann's figures ([1] p. 27) for the healthy fowl are:

	Polymorphs.	Monocytes.	Lymphocytes.	Masts.
	33	12.0	53.0	2

Red blood corpuscles were for hen, 2,900,000 per c.mm., up to 4,000,000 per c.mm. in cocks. Leucocytes were about 30,000 per c.mm.

Histologically, the tumour tissue in these thirteen cases did not differ in any essential particular from the description already given for cocks 86 and 84.

The relative benignity of the tumour in these two batches of inoculations, as compared with its behaviour in cocks 86 and 84 from which the inoculating material was obtained, is striking. Concurrently with this is the less striking monocytic reaction in the blood of the former as compared with that of the latter. In both, however, it occurs and seems to be an essential phase of the process. It is not a defensive reaction, as, if it were, the condition of matters would surely have been reversed.

The nature of the process at the basis of the haemorrhages in this condition could not be determined from cocks 86 and 84. In cocks 20 and 88, the tumour process being less active, its nature was more evident. It seems to consist of a weakening and disappearance of the blood vessel wall, round which the tumour proliferation originated as a perivascular process.

Attention is directed to the essentially nodular growth of the tumour, first, in advance of the main tumour mass in the subcutaneous tumour, and second, in the secondary deposits. A large tumour mass arises by an extension radially of these masses and subsequent confluence. Histologically, these nodules are seen to be developing round small arteries.[1] The enormous numbers of these separate secondary foci, which may occur in an organ such as the liver and lung, renders the idea of their being true metastases very unlikely. A more feasible view is that they are local manifestations of a general systemic disease involving the reticulo-endothelial portion of the haemotopoietic tissues. For the tumour as noted above consists of free-histiocytes, on the one hand, changing over, on the other, into stellate

[1] Marchand, one of the original discoverers of the cells implicated here, noted their marked occurrence in the adventitia of blood vessels. (*Verhand. d. Deutsch. Path. Gesellsch.* 1901, iv. 124.)

and spindle cells, which are really modified fibroblasts. It would seem, however, that the stimulus to the reticulo-endothelial system may not be limited to it alone, but may spread wider to the haematopoietic system in general. Thus, in cocks 86 and 84, and also in cock 88, one finds marked proliferation of the histioblasts, myelocytes, etc., of the periportal tissue of the liver. This occurrence is not limited to experimentally inoculated cases: it will be found frequently in the spontaneous cases to be described subsequently.

The absence of anything in the nature of divisional phenomena in the spindle and stellate cells was very striking. Mitotic figures were most often observed in large dropsical-looking free-histiocytes—(macrophages). It occurred too in the smaller varieties of the same cell. Histioblasts which occurred in the body of the tumour in nests, as also in the vessel walls, added to the growth of the tumour by becoming free-histiocytes or by dividing amitotically.

SUMMARY

Evidence produced in this chapter tends to suggest the possibility that Rous No. 1 tumour is essentially a local manifestation of disease of the reticulo-endothelial system. The essential cell involved would appear to be the free-histiocyte or monocyte. The perivascular tissues are a great storehouse of these elements, and perivascular phenomena would appear to play a great part in the spread.

Other lineages of the reticulo-endothelial system, besides the monocytic one, are affected secondarily, giving rise to a leucotic picture.

III

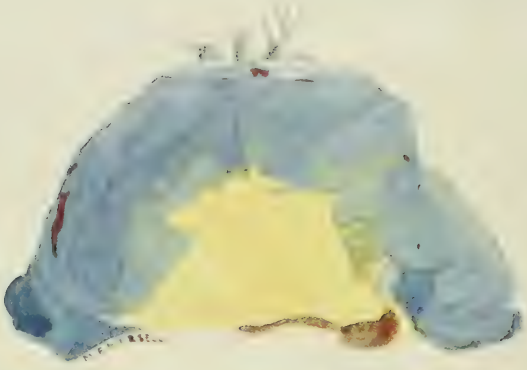
INTRA-VITAL STAINING OF ROUS TUMOUR No. 1

THERE is a considerable body of work dealing with intra-vital staining in physiological conditions. Goldmann (1) initiated and made wide use of the modern dyestuffs by this method. By means of it, he differentiated Kupffer cells (p. 151), reticulum cells of lymph glands, spleen and bone marrow, the inter-stitial cells of the testicle, the follicular cell in the Graafian follicle, and the cortex cell of the suprarenal and the epithelial lining of the convoluted tubules of the kidney.[1] Kiyono (2), later, made an extended systematic survey of the tissues of the body and classified them according to their reactions to such dyestuffs as pyrrhol blue, trypan blue, etc. By means of this technique, dye granules appear in certain cells of the connective tissue series, in consequence of which these cells can be distinguished at once from most of the parenchymatous cells, from the ordinary blood cells, myeloid as well as lymphoid, from the lymphocyte of the lymph nodes and from the plasma cells and mast cells. These granules are of variable size and stain with varying degree of intensity. Arranged according to the fineness and compactness of their granules, an ascending series of vitally staining mesenchymal elements can be tabulated as follows :

(1) The endothelial cells of the blood and lymph vessels. They take the dye only when the staining has been carried to an advanced degree and only in the form of the very finest granules.

[1] It is to be noted at the outset that cells other than those of the R.E. system may contain the dye.

PLATE I.



[*To face page* 21.

(2) The fibrocytes and ordinary connective tissue cells. They store the dye in variable degree, after sufficiently prolonged staining, also in the form of rather fine granules. They are more easily stained than the endothelial cells.

(3) The reticulum cells of the splenic pulp, the cortical nodules and the pulp cords of the lymph nodes, and, ultimately, of the remainder of the lymphoid apparatus. These cells readily take the dye and stain more deeply than the connective tissue cells, but in the rapidity and intensity of the stain fall behind the members of the following groups :

(4) The reticulo-endothelial cells of the sinuses of the lymph nodes, the blood sinuses of the spleen, the capillaries of the liver lobules (Kupffer's stellate cells), the capillaries of the bone marrow, the adrenal cortex and the hypophysis.

(5) The histiocytes, as Aschoff has designated the wandering cells of the connective tissue, the clasmatocytes of Ranvier, etc., to distinguish them from the cells that give rise to connective tissue—the fibroblasts or fibrocytes. These cells stain almost as readily as those of group (4), especially when in a state of heightened activity.

(6) The splenocytes and vitally staining monocytes—blood histiocytes—which have their origin from the histiocytes (group 5), and the reticulo-endothelial cells (group 4).

Aschoff (p. 10) combines groups 3 and 4 under the term reticulo-endothelial system, while Kiyono joins groups 5 and 6 under the term histiocytic elements. Translated into the terms of the blood scheme given on page (2), Aschoff's reticulo-endothelial system is equivalent to the fixed-histiocyte used there, while Kiyono's histiocytic elements are the free-histiocytes or monocytes of the table. The fibrocytes or fibroblasts of group 2 are the fibroblasts of the diagram.

It was thought that, in Rous No. 1 tumour which gave evidence of being of a free-histiocytic derivation, intra-vital staining might clear up certain points. Hence certain of the inoculated cocks—cocks 85, 17, 18, 81, 87, 21, 20, and 90—

FIG. 1.—Cock 21, inoculation with Rous tumour subcutaneously : local tumour : Trypan blue injection, showing necrotic yellow portion in centre, green intermediate portion, and blue ' healthy ' portion, the latter due to the taking up of the dye by the free-histiocytes and to a lesser degree by the fibroblasts.

mentioned in Chapter II, were inoculated with trypan blue. Goldmann (*loc. cit.*) gives as the dosage for small animals a cubic centimetre subcutaneously of a 1 per cent. solution for every twenty grammes of the animal's body weight. He states that this dose can be repeated several times once a week. In larger animals he recommends intra-peritoneal injections, whilst, in others, intravenous dosage may be employed. On the whole he favours subcutaneous administration.

There were no figures to guide one in relation to the administration in fowls. Graduation of the dose according to the animal's body weight as recommended by Goldmann seemed impossible owing to the bulk ; and smaller bulks of more concentrated solutions could not be used owing to the insolubility of the dye.

In any case this did not matter, for the giving of much smaller doses than indicated by Goldmann produced poisonous effects. Two samples of trypan blue—one British, the other German—were used, and the results were the same for both. Thus, cock 85 got 20 c.c.s of a 1 per cent. solution intraperitoneally and died in two days. The intraperitoneal method was therefore abandoned and the subcutaneous substituted. Cock 17 got 20 c.c.s 1 per cent. solution subcutaneously at 10.15 a.m. and again 20 c.c.s at 2.30 p.m. and died next day. Smaller doses were then resorted to and 10 c.c.s of a 1 per cent. solution were administered subcutaneously daily as long as the bird would stand it : generally after three or four injections the animal became very ill and droopy.

The main facts with regard to the tumour growth in the cocks have been already detailed in Chapter II. The reactions of the animals to the trypan blue will alone be dealt with here.

In all the birds, a short time after injection, the beak and scaly part of the legs became a dirty blue, the comb and wattles became livid, the conjunctiva, the mucous membrane of the mouth and the skin, a deep blue. The faeces were blue in colour. The monocytes in the circulating blood showed blue granules in their protoplasm. On *post mortem* examination, the following structures showed blue—the

skin, the buccal mucous membrane, the whole intestinal tract from the duodenum downwards, the ligaments and articular surfaces of joints, the periosteum of bones and fibrous tissue bands between muscles. The liver, spleen, and kidney were of a livid colour—in addition, the kidney showed a blue damascened pattern. The ovary was deep blue in hens, the testicle, on the other hand, showed scarcely any colour. The musculature of the body and the heart were untinged except for some blue streaks on the auricles of the heart. In some cases, the lungs were coloured, in others not.

As a result of the dye, the cellular elements of the tumour could be divided into two groups, a fibroblastic, corresponding to Aschoff's group 2 and a free-histiocytic to his group 5. The former were spindle and stellate shaped and took the dye in very fine granules : the latter were rounded and the dye granules in them were numerous and of large size. Macroscopically, the former corresponded to light blue, the latter to deep blue areas. Necrotic portions appeared yellow, as they took no stain, while necrobiotic fibroblastic areas of the tumour assumed a green tint.

A correspondence was also found between the malignancy, as judged of by local infiltration and secondary growths, and the colouration. Of the eight fowls, six showed a localised, non-infiltrating, freely movable tumour without secondary deposits. Related to this was the fact that the tumours in these cases were deep blue in colour. On the other hand in cocks 20 and 87, the local tumours were infiltrating and secondary tumours occurred ; while the tumour areas were all of a much lighter colour. This would seem to indicate that the malignancy is in some way or another bound up with the appearance of the fibroblast, for as already mentioned, the colour is an index of the type of cell, fibroblast or free-histiocyte present. As will be seen later, however, in cases of spontaneous occurrence of the tumour, this malignancy gives place to benignity when the tumour takes on the form of a dense well-formed fibroma.

It is possible that in this case the malignancy may be

regarded from a purely physical point of view. An exudate of amoeboid free-histiocytes would be little better than a fluid and would be liable to be swept away: fibroblasts, with their interlacing, give solidity, resist removal, and favour infiltration. On the other hand, the dense structure of a hard fibroma confines the elements and offers resistance to infiltration. Carrel and Ebeling's (3) failure to obtain monocytic tissues in fluid cultures are of interest in this connection.

The cases just described show the differentiation of the tumour elements artificially by means of a dyestuff. Instances will be recorded later where, in typical Rous tumours of spontaneous origin, the cell elements are separated out into the two groups by a natural pigment, melanin.

SUMMARY

Intravital staining would seem to show that Rous No. 1 sarcoma is an affection of the free-histiocytic portion of the reticulo-endothelial system. It differentiates the tumour elements into two types, the formation of one of which, the fibroblast, seems to determine the malignancy.

IV

SPONTANEOUS TUMOURS OF THE ROUS TYPE

In this chapter will be described a number of cases of tumours of the Rous type which have been met with in fowls over a period of years. Many of these cases will raise the question of the nature of certain lymphoid cells. It may be stated here, in anticipation, that there is no evidence that the tumours to be described have, in part or in whole, a lymphocytic basis. What appear to be lymphocytes would seem to be in reality small free-histiocytes or in certain cases and localities myeloblasts. The subject will be treated on this basis. The whole question, however, of the lymphoid cell with special reference to the lymphocyte will be discussed in a later chapter.

The present group of cases is one where the Rous type of tumour predominates, but there also occurs in most of them as a secondary phenomenon many of the lesions of a myeloid leucosis. In the next chapter, on the other hand, a group of cases will be described with myeloid leucosis lesions predominating.

Case 1.— 21st February, 1927. Hen 175 : black leghorn ; found dead : two years old : thymus small : lungs, both sides full of nodular white tumours : liver shows several round, sharply delimited, pale white tumours of size of a pea : spleen, slight enlargement, with small tumour nodules : kidneys, several small white foci in them ; very marked sclerosis of bones, metatarsal bone contains fat only.

Histologically, every periportal space contains a mixture of histioblasts and myelocytes : in many cases these are

mixed with free-histiocytes, which shade off into stellate branching anastomosing cells as they creep up between the liver cell columns. These free-histiocytes in the sinusoids are in many instances directly continuous with areas of ' small round cells,' which are evidently not lymphocytes, but small free-histiocytes. In many of the portal spaces there is a round or oval circumscribed nodule of soft fibroma.

The tumour itself is a soft fibroma of rather denser type than Rous No. 1. It contains a number of blood vessels, round which the tumour tissue is whorled. It also shows here and there nests of histioblasts. The larger tumour masses are formed by confluence of several smaller ones.

The tumour in the kidney shows the same characters as in the liver. In the lung, it is of the same nature and occurs in the interlobular tissue.

In the spleen, the tumour mass is formed by the confluence of smaller tumour masses, arising perivascularly round the small arteries. These consist of irregularly shaped cells with basophil granules in their protoplasm. At the growing edge of these larger tumour masses are to be seen the arteries of the malpighian lobules (ellipsoids) surrounded by cells of the above type with, still further out, an area of histioblasts, ' small round cells ' and stellate cells. These would appear to be the units by the confluence of which the large tumour masses are formed. No myelocytes were seen. Cells with basophil granules in the protoplasm are often to be seen surrounding the splenic arterioles in the normal spleen. No marrow was available for examination as the bones were practically entirely sclerosed.

Case 2.—15th February, 1927. Hen 173; black leghorn, two years old, found dead; had been going about in a shrunken condition for a long time. *Post mortem*, it showed

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FIG. 1.—Cock 83: inoculation with Rous tumour subcutaneously: local tumour: invading edge: Leishman's stain: 'a' healthy connective tissue, 'b' tumour showing numbers of 'small round cells' which are really small free-histiocytes 1/12 oil immersion).

FIG. 2.—Hen 175: spontaneous Rous case: liver: Leishman's stain showing several small foci, 'a' in periportal spaces, which, by coalescence, form large tumour masses such as 'b' (low power).

PLATE II.

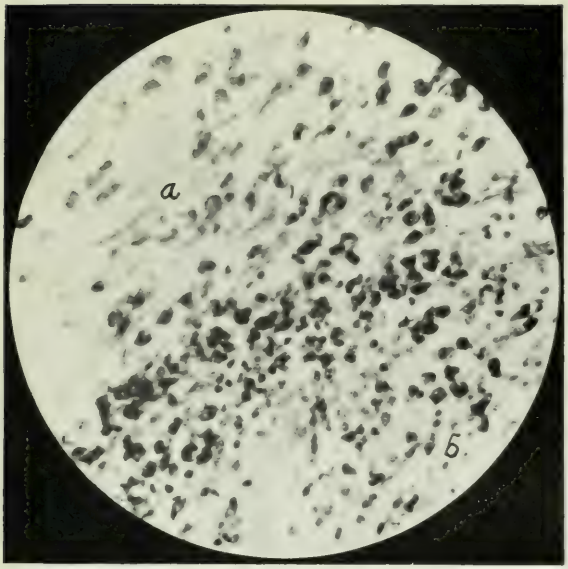

FIG. 1.

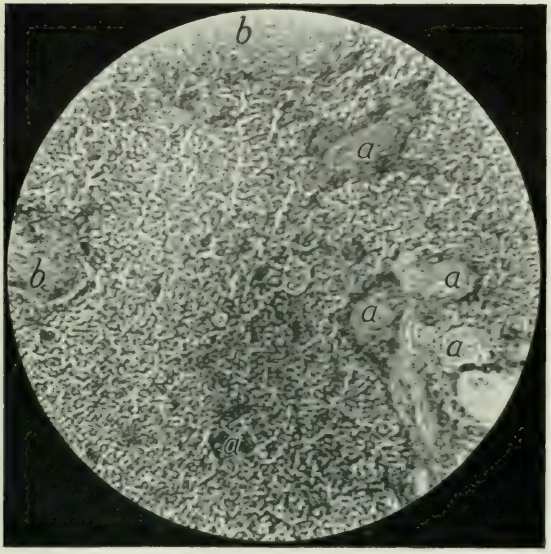

FIG. 2.

[*To face page 26.*

a tumour over the ribs, large tumour in the neck, one on the jaw and another on the outside of the pelvis. The tumour is dense in consistency and tough : the organs showed no marked lesions.

Microscopically, the liver showed slight periportal proliferation of a myeloid type. The tumour consisted of dense whorled fibrous tissue with nests of histioblasts.

Case 3.—9th October, 1926. Hen 140 ; black leghorn, age one and a half years, found dead : thymus enlarged, in pea-like hard nodules : heart shows a large pale white tumour forming the base of the heart and the walls of the auricles : extensive peritonitis, with burst egg yolks, causing matting of intestines : in mesentery, two pale white round tumours of the size of peas : liver, enlarged and full of grey spots : spleen, not enlarged : kidney, pale : in the inner side of muscles of right thigh, a pale white circumscribed tumour of the size of a pigeon's egg, which, on section, shows a somewhat cystic appearance and discharges a mucoid fluid. The marrow of the femur and tibia is greyish and fills the cavity : that of the metatarsal bone is fatty with a pink tinge. The blood films show numerous erythroblasts.

Microscopically, the tumour in the leg, mesentery, heart and thymus is the same. It varies from a loose myxofibroma to, at places, a dense fibroma, showing here and there nests of histioblasts ; sometimes, with myelocytes as in the mesenteric tumour ; the liver shows very marked periportal proliferation of histioblasts and myelocytes, with great destruction of the liver tissue. The spleen shows a condition of the central artery much as that described in hen 175 (Case 1), but without tumour formation. The marrow is very active, with considerable proliferation of haemohistioblasts and myelocytes. The kidney shows areas where the tubules have disappeared while the rest of it has a necrotic appearance.

Case 4.—9th October, 1926. Cock 80, this, an Ancona cockerel (no other was available), six months old, was inoculated over the right breast, with the tumour from hen 140. The tumour slowly increased in size and on 22nd November,

1926, the cock was killed with the object of further inoculations. Parts of the tissue were inoculated into five young leghorn cocks, without result however. No abnormal condition was found in the various organs.

Microscopically, the tumour showed typical Rous structure, with large round dropsical cells, some of them showing mitosis, stellate and spindle cells. There was very marked perivascular infiltration of blood vessels in the surrounding connective tissue with histioblasts and free-histiocytes and ' small round cells.' The body of the tumour showed whorling of the spindle cells, with the remains of arteries and nests of histioblasts in the centre : at places, also dense fibroma structure, with nests of histioblasts : at other places, mucoid degeneration and necrosis.

On the whole, the tumour of this fowl was much denser and microscopically more definitely fibroid than the tumour of hen 140.

There was nothing to note in the liver, spleen, kidney or marrow.

Case 5.—8th April, 1926. Hen 81 : white leghorn : found dead : one year old : shows large tumour on left side over ribs : small white tumours in the substance of the kidney : liver and spleen seem normal : marrow is very active : blood films show nothing abnormal.

Microscopically, the tumour is a soft fibroma of the Rous type with large patches of necrosis : shows numerous large round dropsical free-histiocytes with mitosis. The tumour shows the same characters in all its sites of occurrences. The marrow is very active and myelocytic in type.

Case 6.—26th June, 1926. Hen 112 : black leghorn : found dead : one year old : *post mortem*, enormous liver full of globular white bodies from size of owl's egg downwards : larger ones, slightly necrotic in centre : no tumour in heart :

Fig. 1.—Hen 140 : spontaneous Rous case : dense fibrous tumour in ventricle of heart : Leishman's stain : showing groups of histioblasts ' a ' (characterised by unstained nucleus and deep stained cytoplasm and angular shape) invading between the muscle fibres ' b ' (1/12 oil immersion).

Fig. 2.—Cock 91 : abdominal tumour produced by inoculation of powdered glass Leishman stain : showing interlacing, stellate cells ' b ' forming a meshwork of spaces ' a ' (1/12 oil immersion).

PLATE III.

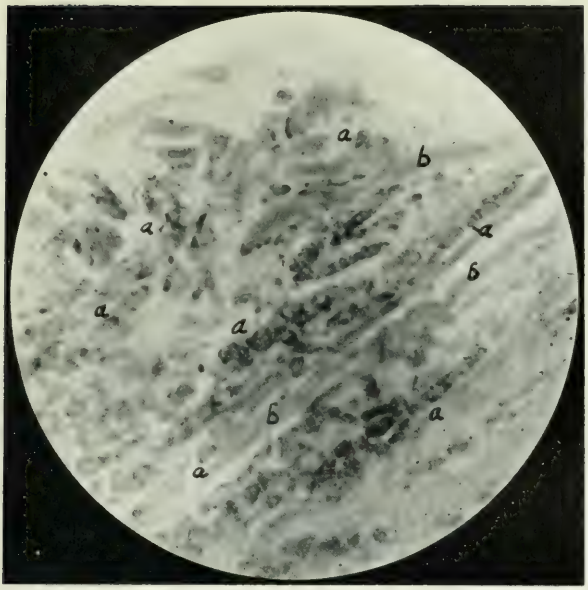

FIG. 1.

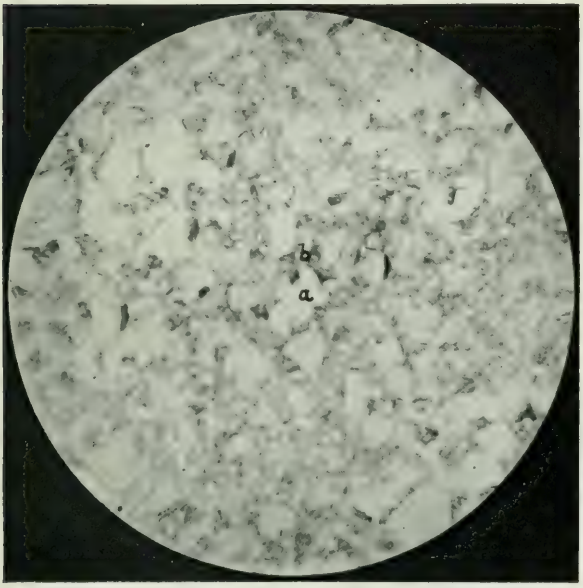

FIG. 2.

[*To face page* 28.

present, in spleen : also markedly, in left kidney : tumour also present in upper end of femur of left side and of tibia of right.

Blood films show nothing abnormal. Microscopically, the tumour is of the typical Rous type in all the situations. In the liver, the periportal proliferation in this case is entirely of the tumour type. In the spleen, the larger masses are necrotic in the centre : they are derived from the confluence of smaller areas, which in turn develop from the sheath of the small arteries.

Case 7.—3rd April, 1926. Hen 88 : white leghorn : found dead : one year old : the lungs are entirely filled with round discrete white masses of tumour from the size of a pea downwards. No tumours seen elsewhere in body. Bones very sclerosed : marrow of metatarsal, red and active : blood films show a few erythroblasts : microscopically, there was nothing to note in the liver, spleen or kidney : the marrow was very active, and myeloblastic : there was marked sclerosis of the bones : the tumour was markedly of the Rous type, with large round dropsical cells undergoing mitosis.

Case 8.—17th May, 1926. Hen 107 : white leghorn : found dead : two years old : showed liver dirty muddy brown with small white spots : tumour-like mass invading left kidney, white in appearance. This tumour mass extends into the left thigh : the bone marrow is fairly red : nothing abnormal noted elsewhere.

Blood films show nothing abnormal. The liver shows marked periportal proliferation of histioblasts, myeloblasts and myelocytes with, at places, tissue resembling Rous tumour : the tumour in kidney and thigh consists of typical Rous tissue with considerable numbers of histioblasts and free-histiocytes : the splenic sinuses are filled with myelocytes : marrow shows considerable myelocytic activity.

Case 9.—14th July, 1925. Hen 19 : white leghorn : haemoglobin, 20 per cent., red blood cells, 750,000 per c.mm. : blood films show large numbers of haemocytoblasts and erythroblasts : liver was not enlarged—appears fairly normal :

spleen, size of owl's egg : large tumour behind rectum of the size of a pigeon's egg : it is adherent to and growing from posterior wall of cloaca.

Microscopically, the tumour is a dense fibroma with numerous islands of histioblasts : the liver shows marked periportal proliferation, in every space, of histioblasts, myeloblasts and myelocytes : there is also a condition of leucostasis here as also in the splenic pulp : the kidney shows intertubular proliferation of histioblasts. The marrow is intensely histioblastic and myeloblastic with islands of myelocytes.

Case 10.— 12th October, 1925. Hen 35 : Rhode Island red : six months old : was discovered with a large tumour on the under surface of the middle of the wing. Haemoglobin 80 per cent. Blood showed nothing abnormal except a leucocytosis : hen died on 17th October, 1925 : *post mortem*, there were large tumours on the left wing, subcutaneously over the right hip and in the lung : microscopically, the tumour was of the typical Rous type, myxomatous in places, at other places dense fibrous : the liver showed periportal proliferation of histioblasts and myelocytes. The marrow was hyperplastic.

This hen, sometime previously, had been mauled by a weasel.

Case 11.—November, 1925. Cock 91 : this was a young white leghorn cock which had been inoculated intraperitoneally sometime previously with a sterile emulsion of powdered glass. When the bird died there was found opposite the needle puncture a large tumour, the size of a Jaffa orange, attached by a slender pedicle to the peritoneal covering of the duodenum. This tumour was of the typical Rous type with spindle and stellate cells, histioblasts and large dropsical histiocytes. There was mucoid degeneration and areas of necrosis.

A special point to note is that it followed on the inoculation of the glass powder and was apparently caused by it. The needle, which was a large one (formed from a small catheter), apparently injured the surface of the duodenum. There was

no sign of an inflammatory condition such as would have followed a puncture of the duodenum itself by the needle.

Case 12.—24th July, 1926. Hen 126 : black leghorn : found dead ; pale comb : haemoglobin 70 per cent. : whole of peritoneum covered with small white tumours size of pin's head up to a pea ; also a large tumour behind the cloaca. No bone sclerosis : marrow, pinkish fat : microscopically, there was nothing to note in the liver, spleen, or kidney : the tumour was a mesoblastic one of modified Rous type. Peritoneal tumours contained in addition some foci of myelocytes : the bone marrow consisted practically of fat only.

Case 13.—1st February, 1927. Hen 167 : white leghorn : found dead : two years old : shows tumours in the infra-orbital region and on both sides of the body over the ribs. The liver is small and studded all over with small white dots. Bones greatly sclerosed. Microscopically, the tumour is a dense fibroma with whorled appearance built round blood spaces. Where the tumour is invading the muscle, the arteries are surrounded by a zone of stellate anastomosing cells. In the liver there is marked periportal proliferation : here the cells are of a stellate anastomosing type. In the spleen, the small arteries are surrounded by an area of the same type of cell. The marrow remaining shows a myelocytic hyperplasia.

The main facts regarding a series of fowls with spontaneous tumours of the Rous type have been described. The following points seem worthy of attention :

The tumours themselves have shown a wide variation in the development of the constituent cells. At one end of the series the tumours have been soft in consistency, a condition associated with cells of more embryonic type. At the other end they have been hard and the tissue of the nature of fibrous tissue. The former type appeared to be more malignant, invading locally more freely and with a greater tendency to secondary growths. These conditions were seen in cock 80 as compared with hen 140 which supplied the tumour for inoculation. Both types were associated with perivascular

spread in front of the main body of the tumour. In addition to the spindle and fibrous tissue elements, both showed free-histiocytes and nests of histioblasts.

A considerable proportion of them, while showing no marked changes in the circulating blood, showed yet marked periportal proliferation in the liver, and hyperplasia of the marrow and sclerosis of the bones. In several cases, the hyperplasia in the liver was myeloid in type, in others, mixed with tumour cells, whilst in a few, it consisted purely of tumour tissue. In the last type the tumour was of a diffuse nature, insinuating itself from the periportal spaces between the liver lobules throughout the organ. In the spleen, it was present as a sheath surrounding the small arteries. In both types of case, the nature of the proliferation suggested a system disease rather than a metastatic phenomenon. In some cases, the activity was limited to the free histiocytic lineage of the reticulo-endothelial system together with the fibroblast: in others, it overflowed and affected other lineages, notably the myelocytic.

In only one case was the thymus enlarged—hen 140. This was due to tumour growth. The bursa of Fabricius in two cases was attacked.

In one case there was an actual growth of the tumour in the marrow.

It is not proposed at this stage to discuss the question of the ultimate cause. Certain features regarding possible precipitating forces may, however, be mentioned. Thus, the subcutaneous position of many of what appeared to be the primary lesions lends colour to the idea that they may have resulted from injury. In this regard, hen 35 may be specially mentioned where the primary lesions were undoubtedly on the middle of the wing and over the rump, in both cases subcutaneous. Here, the lesions were due to the bite of a weasel. It is not suggested that anything of the nature of rat-bite-fever as it occurs in human beings, with its spirochaete causation, was produced. Even in humans, however, ill-defined tumour conditions, without the clinical syndrome of typical rat-bite-fever, have resulted

from bites of weasels, ferrets, cats, etc. All that is mooted is that some non-specific inflammatory action, due to some organism or other, ended up in the appearance of the tumour. Again, cock 80 deserves consideration in this connection. Here the tumour appeared subsequent to the inoculation of sterile powdered glass into the peritoneal cavity, with probable injury of the peritoneal surface of the duodenum. The needle might have penetrated the duodenum, raising the question of possible infectious origin of the tumour. It is more likely, however, that in such a case acute peritonitis would have been the result.

The discussion of cases, where the primary tumour seemed to arise in the kidney (hens 101 and 112), in the lung (hen 88 and hen 175), will be left over meantime. The occurrence of a local irritation of possibly diverse origin setting the hyperplasia going is even here not a forced conception.

SUMMARY

The evidence obtained from spontaneous tumours would seem to support the tentative conclusions already arrived at in Chapters II and III. A series of cases have been described in which a Rous type of tumour is associated secondarily with leucotic phenomena of a myeloid type.

It is indicated that irritants of a non-specific type may possibly play a part in the origination of the tumour process.

V

LEUCOTIC TUMOURS IN FOWLS

The subject of this chapter may be made clearer by a reference to the diagram in Fig. 1. There, the haemohistioblast will be seen giving rise to the histioblast which in turn produces the lymphocyte, the myelocyte and the monocyte —the latter giving rise in turn to the blood vessel endothelium and the fibroblasts. With the lymphocytic lineage we have nothing to do at present: the myelocytic and the monocytic alone concern us here. If one considers the possibility of tumour formation in these two lineages, it has already been shown that purely monocytic tumours have not been found. Possibly this may be associated with the motility [1] of the monocyte, as tumours of polymorphonuclear leucocytes do not occur, persumably for the same reason. Fibromas of the Rous type have already been discussed and endotheliomas occur, if not in the fowl, at least in mammals. It has been shown, too, that fibromata of the Rous type in fowls are in very many cases associated with leucotic phenomena as a secondary lesion.

In this chapter will be described tumours in the fowl belonging to the myeloid lineage and to cell types of intermediate stages in this. Thus, myelocytic and myeloblastic growths occur which can easily be identified as such. With regard to the histioblastic and plasma cell tumours which occur, difficulties may be met with in the assigning a definite

[1] Carrel and Ebeling's (1) findings with regard to monocytes when grown in fluid cultures are of import. In such a case the monocytes wander about and never form a tissue.

lineage to them. The occurrence of myelocytes somewhere in the tumours with the absence of anything of the nature of cells of the Rous type help to settle this question. As a matter of fact the majority of the tumours to be discussed here are preponderatingly myeloid in type.

Only cases with definite tumour formation are treated of here. This leaves out of consideration a large number of cases met with in which the leucotic proliferation was of a diffuse nature not amounting to tumour nodules.

Myelocytomas (one case).

Case 1.—8th September, 1925. Hen 45 : white leghorn : one year old : haemoglobin 70 per cent. : red blood cells, 3,000,000 per c.mm.

19th November, 1925, hen dying : haemoglobin 70 per cent. : blood films show numerous myelocytes and myeloblasts. *Post mortem*, there was found a chalky white tumour, soft in consistence, involving the kidney fossa on left side, the ovary, and the inside of the lower ribs. The marrow was of a red colour. The liver showed minute white spots all over : spleen, kidney, and thymus appeared normal. Microscopically, the tumour consisted of a mass of pure myelocytes, with many mitotic figures : the marrow was a mass of myelocytes, and myeloblasts. The liver showed extensive periportal proliferation of myelocytes and myeloblasts. Nothing abnormal in spleen or kidney.

Presumably, in this case, the tumour arose in the ovary, which, as will be shown later, contains tissue with myeloid potencies in the fowl.

Myeloblastomas (two cases).

Case 1.—31st March, 1927. Hen 188 : white leghorn : blood film shows marked myeloblasts and a few normoblasts.

Post mortem, the liver was of enormous size with small white dots and also several large white and brown tumours : the spleen was the size of a hen's egg : the kidneys were enlarged and of a solid fleshy look : the bones were entirely sclerosed, except for a small amount of pale marrow in the centre of the tibia.

Microscopically, the liver tissue is nearly wholly destroyed and its place taken by tumour growth of myeloblasts with myelocytes. The splenic pulp was choked with myeloblasts with a few myelocytes. The kidney showed extensive intertubular myeloblastic proliferation. The marrow was practically entirely myeloblastic and myelocytic.

Case 2.—18th June, 1925. Hen 16: this hen showed practically identical conditions as in hen 188, with the addition of patches of histioblasts at places in the liver tumour.

Plasma cell tumours (five cases).

Case 1.—3rd January, 1927. Hen 158: white leghorn: eight months old: found dead: the liver was enormous and full of small and large white tumours: the kidneys were enlarged and very dark in colour: the marrow was dark red: the marrow of the metatarsal bone instead of being yellow fat as usual was also dark red. Nothing abnormal was found in the blood films. Microscopically, the liver tissue was nearly all destroyed by extensive periportal tumour tissue. The cells constituting this tissue were not histioblasts nor were they myeloblasts. They were of the plasma cell type with a halo round the nucleus and irregular masses of basophil substance in the outer portion of their cytoplasm. Many of these cells are intermediate stages, as described above, between the histioblast and the myelocyte: others of them undoubtedly represent degeneration stages of the histioblast on the way to become the 'small dark cell.' Intermingled with them were numerous 'small dark cells,' which, as just mentioned, are probably aborting histioblasts (2). A few myeloblasts and myelocytes were also present. The central artery of the malpighian lobules (ellipsoids) of the spleen were surrounded by a thick zone of plasma cells. The spleen was not enlarged. The kidney was very vascular: at places it was necrotic: it contained masses of the tumour with the same type of cells as in the

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FIG. 1.—Hen 45: myelocytoma, sporadic case. Leishman's stain; tumour consists entirely of myelocytes (1/12 oil immersion).

FIG. 2.—Hen 158: plasma celled tumour: sporadic case. Leishman's stain: tissue consists almost entirely of plasma cells, as at 'a' with deep stained nucleus, unstained halo of cytoplasm round it, fringed by a deep staining border (1/12 oil immersion).

PLATE IV.

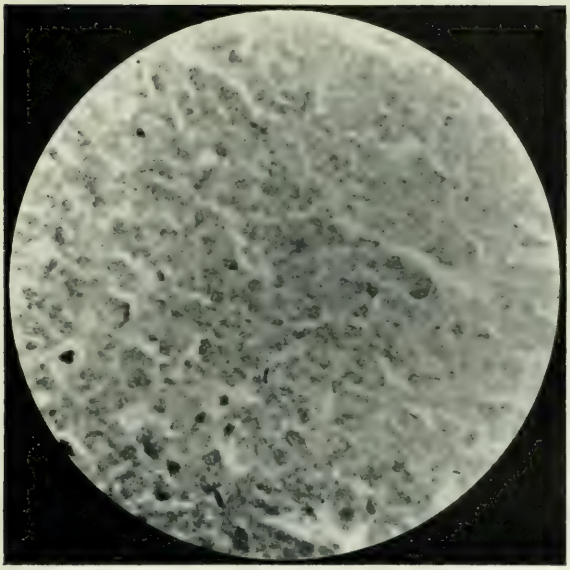

FIG. 1.

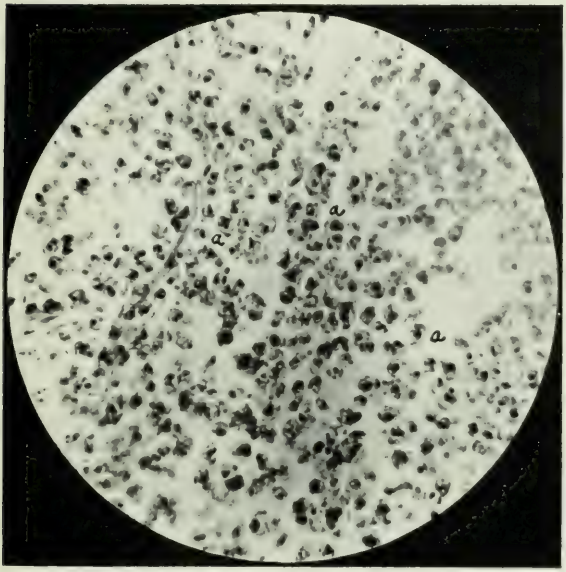

FIG. 2.

[*To face page 30*

liver. The marrow showed myeloid hyperplasia, with myelocytes, myeloblasts, plasma cells and ' small dark cells.'

Case 2.—17th January, 1927. Hen 161 : white leghorn : two years old : blood film shows marked erythroblastic and myeloblastic changes : liver enlarged and spotted : spleen slightly enlarged : kidney enlarged and pale : bones sclerosed : marrow very pale. Microscopically, the liver shows periportal proliferation in every space of myelocytes and plasma cells together with ' small dark cells.' The kidney shows interstitial proliferation of the same type of cells. The marrow is hyperplastic and shows the same type of cells.

Case 3.—15th April, 1927. Hen 193 : white leghorn : one year old : nothing found in blood film made *post mortem* : liver of enormous size with numerous white nodules in it : spleen enlarged four times : kidneys enormously enlarged on both sides and pale white : large pale tumour behind cloaca : bones not sclerosed : marrow dark—metatarsal bone shows fat only. Microscopically, the conditions present were as in hen 158 (Case 1). The post-cloacal tumour consisted of plasma cells and ' small dark cells.'

Case 4.—9th April, 1925. Hen 15 : the liver only was obtained from this case. It was enormously enlarged, and had the same macroscopic and microscopic appearance as in hens 158 and 193.

Case 5.—28th January, 1927. Hen 164 : white leghorn : one year old : very thin : enormous liver with white tumour masses all through it. Kidneys, large white tumour masses : spleen not enlarged : bones sclerosed : marrow greyish red. The microscopic findings were much as in the previous four hens : they differed, however, in this respect, that typical histioblasts formed a larger proportion of the tumour cells, which paves the way for a discussion of the next group of cases.

Histioblastomas (four cases).

Case 1.—17th July, 1926. Hen 120 : black leghorn : one year old : blood film shows nothing abnormal : liver,

greatly enlarged with white dots in it : spleen, enlarged three times : kidneys, enormously enlarged : post-cloacal tumour of the size of a pigeon's egg : bones, not sclerosed : marrow, grey pink : metatarsal bone shows fat only.

Microscopically, the tumour cells in all the organs consisted practically entirely of well-formed histioblasts. The liver tissue was nearly all destroyed by them, as also occurred in the case of the kidney. The marrow showed marked hyperplasia of histioblasts, myeloblasts and myelocytes. In the spleen, the small arteries were surrounded by a zone of irregularly shaped cells with basophil dots int heir protoplasm, with, further out, a mass of typical histioblasts. The tumour itself consisted, in places, of pure histioblastic tissue : at other places of dense stellate branching interlacing cells with foci of histioblasts and myelocytes : at other places of areas of necrosis.

This case introduces for the first time the difficulty of determining whether the tumour had a free-histiocytic lineage of Rous tumour type in it as well as a myelocytic one.

Case 2.—25th April, 1924. Hen 29 : black leghorn : very pale comb : blood films show considerable number of erythroblasts. *Post mortem*, there was found a large white tumour behind the cloaca and sharply circumscribed white tumours, some of the size of a hazel nut in the liver : the spleen was slightly enlarged : the marrow was not examined : the tumours consisted entirely of histioblasts : the liver showed extensive myeloid proliferation in the periportal spaces.

Case 3.—12th April, 1926. Hen 84 : black leghorn : one year old : liver, slightly enlarged : spleen, enlarged three times : kidneys, large, pale : large tumour, size of Jaffa orange, behind cloaca growing from posterior wall : bones, not sclerosed : marrow, pink : fat only in metatarsal bone :

FIG. 1.—Hen 158 : ' secondary follicle ' of spleen : plasma celled change : sporadic case : Leishman's stain, showing ' a ' circumscribing wall, ' b,' plasma cells (1/12 oil immersion).

FIG. 2.—Hen 120 : histioblastic tumour : sporadic case. Leishman's stain : tissue consists practically of histioblasts of irregular size and angular shape with pale unstained nuclei and deeply stained cytoplasm (1/12 oil immersion).

PLATE V.

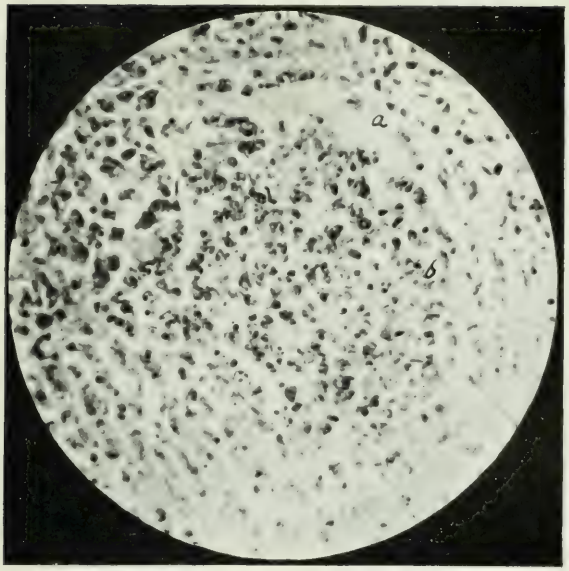

Fig. 1.

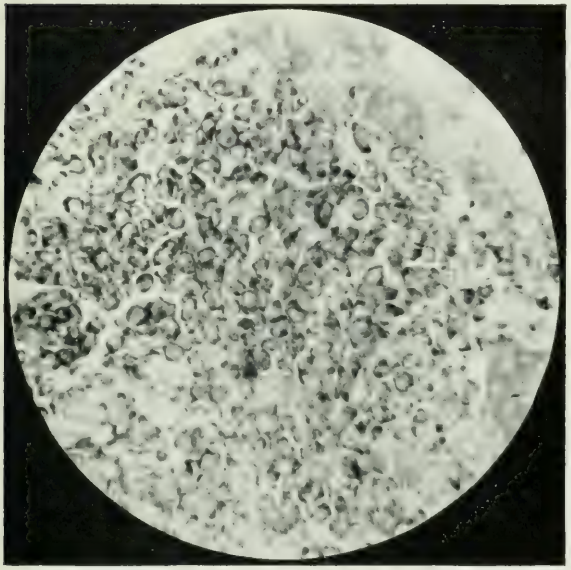

Fig. 2.

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nothing abnormal in blood film. Microscopically, the liver showed small amount of periportal tissue in every space. This consisted of histioblasts, myeloblasts, and myelocytes. The kidney showed histioblastic proliferation between the tubules. The spleen showed nothing marked : the marrow was slightly myelocytic : the tumour itself consists of histioblasts, practically entirely, with a few plasma cells.

Case 4.—25th February, 1924. Hen 10 : was found dead : large tumour, of the size of tangerine orange, present in pelvis : spleen, enormously enlarged : liver, enlarged with white tumours : hen died from haemorrhage of spleen. The tumours present here were histioblastic in type, and in most respects the hen resembled hen 29 above.

Tumours of myelocytic lineage with an element of free-histiocytic (four cases).

Case 1.—30th March, 1926. Hen 74 : white leghorn : found dead. *Post mortem*, showed two tumours at root of tongue ; also ulcerating lump at back of pharynx opposite the opening into the trachea. Thymus, slightly enlarged : liver, slightly enlarged and studded all through with white dots ; also a tumour-like mass at the lower margin : spleen, enlarged three times : kidney, pale : whole intestines matted together with a tumour mass, apparently arising in ovary : no scleroses of bones : marrow, pale : fat only in metatarsal : blood films show a few myelocytes, some normoblasts, and some haemocytoblasts.

Microscopically, the kidney sections showed, at the periphery of the kidney, a fairly large pure focus of myelocytes between the tubules : elsewhere, get intertubular foci of histioblasts giving rise, at places, to myelocytes. The large mass of tumour in the liver consists, at places, purely of histioblasts—at other places, of free-histiocytes, changing into stellate and spindle cells. All over one finds patches of necrosis : elsewhere in the liver get marked periportal proliferation of histioblasts, myelocytes, and at places ' small round cells.' The tongue tumour shows a mixture of patches of histioblasts with areas of myxofibroma. The thymus shows a solid mass of isolated histioblasts changing

at places into myelocytes : the abdominal tumour shows a fibrous tissue capsule, with beneath it patches of myxofibroma at some places, at others, masses of closely packed histioblasts. The marrow shows myeloblastic and myelocytic hyperplasia with areas of histioblasts and ' small dark cells.'

This seems to be a case of a tumour of the Rous type arising from the ovary associated with very pronounced leucotic changes.

Case 2.—11th October, 1926. Hen 141 : white leghorn : six months old : very emaciated : solid grey tumour in lungs : liver, slightly enlarged : spleen, small : kidneys, numerous white circumscribed tumours : bones, no sclerosis : marrow, pink : blood films show erythroblasts. Microscopically, the kidney sections show the kidney tissue destroyed and displaced by a tissue consisting of histioblasts, changing into myeloblasts and myelocytes : same type of cells surround artery in spleen : the lung tumour tissue consists practically entirely of stellate branching cells of the Rous type : liver shows no periportal proliferation. The marrow shows intense myelocytic proliferation.

Case 3.—14th July, 1926. Hen 119 : black leghorn : three years old : haemoglobin 70 per cent. : blood film shows slight erythroblastic changes : enormous size of liver with tumours varying in size from that of a pea to a marble. Spleen, much enlarged ; on one side of it, a large white tumour : kidneys, enlarged and pale. Microscopically, the liver shows nearly the whole liver tissue destroyed by periportal proliferation of histioblasts, myeloblasts and myelocytes : the kidney showed the same type of cellular infiltration : in the spleen, the tumour consists, at places, of histioblasts, becoming changed into stellate anastomosing cells : at other places, almost entirely of stellate anastomosing cells of the Rous type, with here and there foci of histioblasts : the bones show great sclerosis : the marrow is histioblastic.

Case 4.—2nd August, 1927. Ancona : two years old : very emaciated : blood, very watery : films show numerous

erythroblasts : liver, slightly enlarged, pale brown—shows small white dots : spleen, very small : kidney, enlarged, pale : bones, very sclerosed : marrow, very pale : a small pale tumour on the outside of the thigh muscles.

Microscopically, the liver shows marked periportal proliferation of myelocytes and myeloblasts : the marrow is extremely aplastic : the tumour in the muscles of the thigh is a myxofibroma of the Rous type. These cases show the association of Rous type of tumour with a proportionately more active hyperplasia of the myeloid tissues than occurred in the cases in Chapter IV.

Tumours of the primitive mesenchyme have not been met with in this series. Goldschmidt and Isaak (3) and Letterer (4) have described, however, in human beings leucaemic proliferations of the reticulo-endothelial-system affecting mainly the reticular components.

The haemohistioblast has twofold potencies, an erythroblastic and a histioblastic one. Nothing corresponding to a haemocytoblastic tumour was found. It is possible that the leucostatic cases of leucosis, which occur where the blood vessels of the liver, spleen, kidney, etc., are distended with haemocytoblasts, approaches this condition. In such cases, the intravascular position of origin and lack of coherence of the cells formed would prevent anything of the nature of a local tumour being formed.

All the tumours met with in this series of cases have been derived directly or indirectly from the histioblast. As already referred to, no lymphoblastic or lymphocytic tumours have been encountered in the fowl. Those present have been associated with the myelocytic and monocytic lineages into which the histioblast otherwise develops.

Attention has been directed to the association of leucotic conditions with Rous tumours. Leucosis may, and usually does occur without any coexistent Rous tumour. On the other hand, if the Rous tumour be at all advanced and, even in early cases, it rarely occurs without concomitant lesions of leucosis. This occurrence is not likely to be due to separate causation of the two conditions. The clinical

observations and especially the results of inoculation of Rous tumours into fowls, as already described, are against such a view.

In both cases, all the evidence is against metastasis being the prime factor in the origination of secondary deposits. Everything points to the conditions being system diseases of the reticulo-endothelial system. It would seem that in the primary lesion, say of leucosis, a specific cell stimulating substance (stimulin) is produced which stimulates the growth of myeloid tissue elsewhere in the body in the domain of the reticulo-endothelial system, wherever the environment of the cells in question is favourable for this growth. The same would appear to hold for the Rous tumour. Here, however, the stimulating agent would have a wider area of influence, regionally speaking, and would also affect more cell lineages. It is well recognised that the free-histiocyte (or its immediate precursor), which is the basic cell of the Rous tumour, is a cell with considerable developmental potentialities and that it is capable of marked dedifferentiation or reversibility. This would serve to explain the stimulation by the agent not only of the Rous cell lineage but of the myeloid lineage as well.

In harmony with this is the distribution of the lesions in the two cases. The leucotic process affected chiefly the marrow, the liver, the kidney and the spleen, more or less in this order of frequency. The lung and the subcutaneous tissues were not implicated. The Rous tumour, on the other hand, had a predilection for the subcutaneous tissues, the kidney, the lungs, the liver, the spleen and the heart, in this order. In one case also, the marrow was affected. The Bursa of Fabricius seemed to be a site where both types of tumour could occur, as was also the ovarian stroma. Both these tissues contain latent myeloid cells in addition to the ordinary reticulo-endothelial elements. The more limited distribution of the myeloid growths may be noted.

The great majority of leucotic tumours in the fowl are unassociated with any change in the blood in the direction of a leucaemia, etc. In this respect, therefore, they do not

PLATE VI.

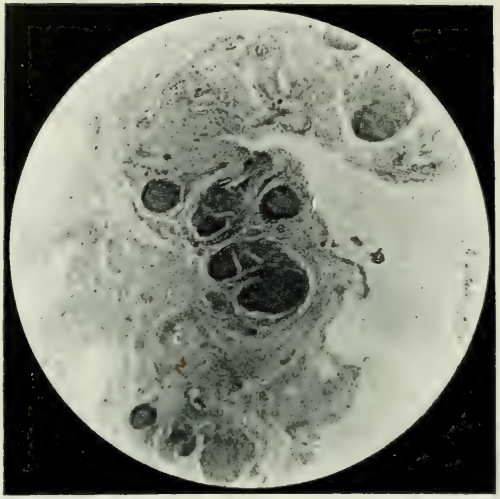

FIG. 1.

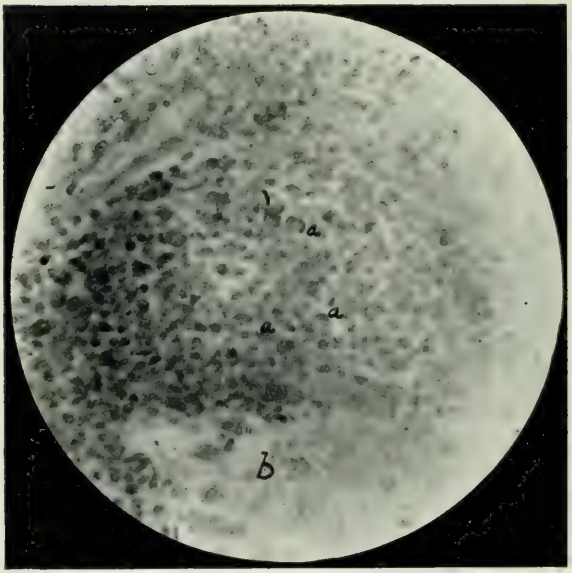

FIG. 2.

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differ from ordinary sarcomatous tumours and, in considering them, it should be forgotten for the moment that the tissues, from which they arise in ordinary circumstances, supply certain cells to the blood. In such cases, the idea of a demand by the blood for more cells being eliminated as a cause of the hyperplasia, effort can be directed to comparing these tumours with other sarcomatous tumours on a common basis.

In human leucotic conditions, of course, a leucaemia of the blood is the rule. The difference in the two species might be explained thus. In the fowl, intravascularity and extravascularity are terms to be rigidly interpreted. In man, on the other hand, intravascularity, while still logically remaining such, has partaken somewhat of the nature of extravascularity, and, conversely, extravascularity has gained something of intravascular character. The result is that there is an easier access of extravascular cells, especially when deficient in motility, to the blood stream.

The rapid growing leucotic tumours and the Rous tumours exhibit all the characters of malignancy. Indeed, it may be argued that they may be more malignant even than the tumours usually so-called. They exhibit the ordinary infiltrative powers, together with the destruction of the infiltrated tissues. If metastasis occurs with them, its occurrence is overshadowed and concealed by the much greater systemic spread. This systemic spread is really only the local spread writ large. It is a characteristic, possessed by these tumours, which other ordinary sarcomata may not have, although Jolly has hinted (5) at this possibility. It is an essential feature of these tumours and adds thereby to their malignancy. Spread by metastasis, as understood for other tumours, is a fortuitous circumstance, depending on the accident of cells entering a vessel or being phagocyted and deposited in a lymph gland. There is no evidence that this property is not equally possessed by the leucotic and Rous tumours. It seems, therefore,

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FIG. 1.—Cock 80: inoculation case: local tumour. Leishman's stain: showing perivascular spread of the tumour, at a distance from the main mass (low power).

FIG. 2.—Cock 80: one of nodules from above magnified, showing characteristic histioblasts, 'a' among more numerous free-histiocytes: 'b' is the lumen of the artery (1/12 oil immersion).

that these latter tumours possess, in superabundance, the qualities that make for malignancy. It should be noted further that, in regard to leucosis, the leucotic tumours are only the top members of a series, grading down, by intermediate stages, to ordinary leucosis with its diffuse infiltrations.

Leucotic tumours, like ordinary mesenchyme tumours, may be simple or malignant. This depends on the developmental stage of the constituent cell. The more embryonic this is, the greater is the malignancy. They differ however from ordinary tumours in possessing the system spread. Hence the greater malignancy of the malignant types.

It will have been observed that in the present treatment of the subject, Rous tumours are regarded as leucoses, which in turn are considered to be essentially tumours.

SUMMARY

Leucotic tumours of various types as occurring in the fowl have been discussed and their derivation from various cells in the myeloid lineage pointed out. The association of Rous tumours with leucaemic phenomena has been emphasised as has also the fact that both are system diseases of the reticulo-endothelial system.

Rous tumours and malignant leucotic tumours are to be regarded as malignant growths of enhanced malignancy.

A reason for the continued growth, subsequent to the appearance of the primary growth, has been suggested in the appearance of a specific stimulin.

VI

MELANOMATA IN FOWLS

In this chapter, the subject of melanomata will be discussed, the treatment being based on some cases which have been met with in the fowl. Some of them are essentially of the Rous sarcoma type.

Case 1.—8th February, 1927. Hen 172 : black leghorn : two years old : very emaciated : comb shrunk : blood film shows nothing abnormal : there is a large black lobulated tumour of the ovary. The whole of the peritoneal surface is studded with irregularly sized tumours, round in shape. Most of these are deep black in colour but a few are white or partly white : nothing particular of note was found elsewhere in the body.

Microscopically, the tumour is a mesoblastic one, of exactly the histiological appearance already described for Rous tumours. In addition, the granules of melanin showed the same distribution that has already been described for trypan blue granules in Rous tumour fowls injected with trypan blue. The free-histiocytes were crammed with numerous large granules, while the fibroblasts showed few and small ones. The marrow was very myeloblastic and myelocytic.

Case 2.—8th March, 1927. Hen 180 : black leghorn : two years old : died suddenly : muscular and very fat : blood film shows marked erythroblastosis : ovary shows a mixture of large yolks and round black tumours. The peritoneal surface is covered with numerous round tumours, mostly small and black, but some white or mixed with white : nothing abnormal found in liver, spleen, or kidney : marked

sclerosis of bones. Microscopically, the condition present here in the tumour is the same as in hen 172, with, in addition, at places, large foci of myelocytes. Sections of the bone showed the sclerosis, with marrow of the myelocytic type : small patches of the tumour, with melanin pigment, in the interlobular spaces of the lung.

In both these cases, there is a tumour of the ovary of a mesoblastic type, the cells of which contain melanin. In the next case, the tumour cells are epithelial in character, while at the same time containing melanin.

Case 3.—26th May, 1927. Hen 204 : Rhode Island Red : nothing found in blood films : liver, not enlarged, but shows white streaks in it, also two small round white tumour nodules. The whole of the intestinal viscera are matted together with masses of tumour, the origin of which appears to be the ovary. The peritoneal surface is also covered with small tumour nodules. On section many of these tumours show a yellow centre, like yolk of egg ; also here and there patches of a black colour are found : nothing abnormal in spleen or kidney : the bones are greatly distended and the cortical bone is no thicker than a sheet of paper : the marrow is of a deep blood red colour.

Microscopically, the tumour consists of collections of epithelial cells which have proliferated from the membrana granulosa into the Graafian follicle. This appearance is present both in the ovary and in the secondary tumours. Most of the cells are filled with large globules of fat : in the dark patches, these epithelial cells contain melanin. The nodules in the liver consist of this epithelial tumour. No evidence of any sarcoma of the stroma. The marrow shows marked hyperplasia.

These cases of tumour of the ovary have occurred in coloured fowls. The next two were found in albino fowls.

Fig. 1.—Cock 80 : early perivascular spread. Leishman's stain : showing, 'a,' the lumen of small artery, 'b,' collection of free histiocytes with a few histioblasts, 'c,' connective tissue (high power).

Fig. 2.—Hen 209 : ovarian tumour : sporadic case : of Rous type, Leishman's stain : showing whorled arrangement, due to the development of the fibrous tissue elements from the theca interna of the egg follicle (low power).

PLATE VII.

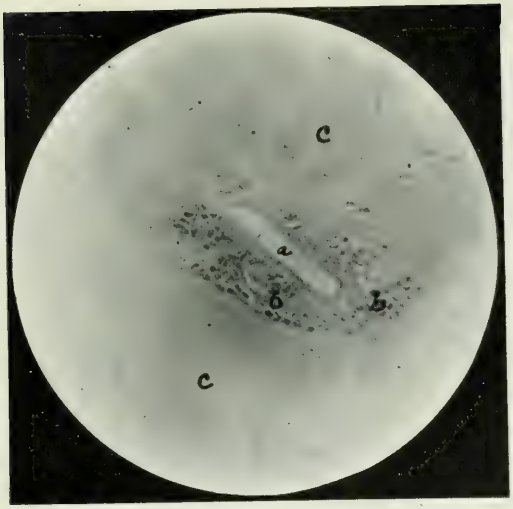

FIG. 1

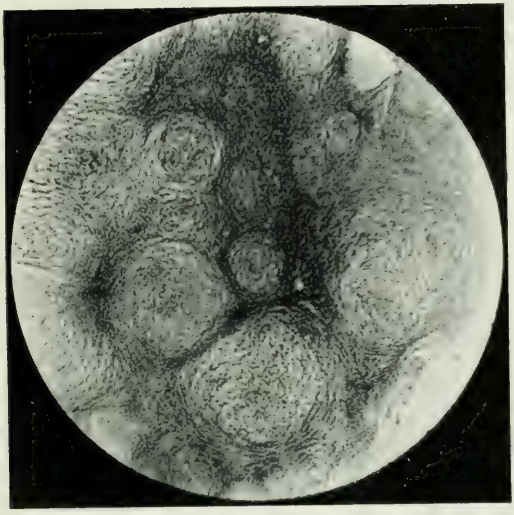

FIG. 2.

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Case 4.—30th March, 1926. Hen 74 : white leghorn with Rous tumour of ovary : already described (p. 39).

Case 5.—18th January, 1927. Hen 202 : white leghorn : two years old : it showed peritonitis with burst egg yolks : large white tumour in ovary and also small nodules all over the peritoneal surface : liver shows pale white spots in it : these also occur in kidney : bone marrow is reddish with pale spots in it. The blood film shows nothing abnormal.

Microscopically, the ovarian tumour is of the typical Rous type but shows also large foci of myelocytes and myeloblasts. The kidney shows extensive intertubular growth of myelocytes. The liver shows marked periportal growth of myelocytes and myeloblasts, while the marrow shows very marked myelocytic transformation.[1]

The pigment in the melanotic cases was subjected to the tests for pigments set forth in Hueck's Table (1) and gave all the reactions of melanin. It should be particularly noted that no pigment occurred in the tumour of hens 74, 202, or 209, *white* leghorns.

The chemistry and mode of formation of melanin are of great interest in connection with the present subject. The following points in connection therewith are derived in the main from Dawson's exhaustive monograph on melanomata (2), or from Spencer's article (3). Melanin is closely allied to adrenalin. It is derived from melanogen, a colourless mother substance, which is, in all probability, a pyrocatechol derivative and is more nearly allied to 3-4 di-hydroxyphenylalanin derived from the broad bean (' dopa ') than any of the other aromatic derivatives of the complex protein molecule. According to Dawson (following Bloch), it undergoes oxidation in the epithelial cells of the epidermis and hair matrix

[1] Since the above was written another hen, hen 209, was obtained on 8th June, 1927, with Rous tumour of the ovary. It was a white leghorn, two years old ; it showed rounded tumours in the ovary and over the peritoneal surface, all pure white. Microscopically, the tumour was of a very whorled type (Fig. 2 Pl. vii). It seems to be growing from the theca interna. The liver showed marked myelocytic periportal proliferation ; the marrow marked myelocytic hyperplasia.

to a black melanin by an oxydase (' dopa ' oxydase) which is an absolutely specifically differentiated product found only in these cells and a few other cells of epidermal origin. Spencer (p. 909), however, does not take up so rigid an attitude, for he thinks that, when melanotic cancer has supervened, the connective tissue cells which have become sarcomatous produce the ' dopa ' oxydase as well

Other forerunners of melanin are indicated by the following. Quattini (quoted by Spencer), injected pyrrole, indol and skatol under the skin of rabbits and got increased growth of pigmented hair, the pigment produced being very like melanin. This result did not follow in albino rabbits or in white spots of other rabbits. Again, if the skin of brown or grey mice were tarred, there resulted a pigmentation of the skin due to melanin, even before cancerous changes were induced. This did not occur in white mice. Pigmentation of a patch of vitiligo resulted from the injection of adrenalin, after the patch had been exposed to ultra-violet rays. Bloch also found that his ' dopa ' oxydase when abundantly present turned hairs brown by acting on adrenalin (Dawson, p. 664). This would seem to indicate that there may be not only a mother substance common to the two but that the one (adrenalin) can be changed directly into the other (melanin). In this connection may be mentioned Jäger's (4) experiment where, with the extract of a melanotic tumour of the horse, he transformed adrenalin into melanin.[1] A solution of melanin (quoted by Spencer) has the same effect on the blood vessels of the frog, only weaker, as one of adrenalin ; on the heart of the rabbit, the melanin produced more effect than the adrenalin. Roaf found that a colourless fluid, secreted from a rectal gland in molluscs and which becomes

[1] Neuberg is quoted by Sharpey-Schafer (vol. i. p. 100) as having obtained the same result.

FIG. 1.—Hen 180 : ovarian melanoma : sporadic case of sarcomatous (Rous) type : unstained : showing, ' a,' fibroblasts with a few granules, of melanin and ' b,' free-histiocytic cells with large melanin granules (low power).

FIG. 2.—Hen 204 : ovarian melanoma : sporadic case of epitheliomatous type. Leishman's stain : epithelial cells arising from zona granulosa of egg follicle : showing, ' a,' epithelial cell mass without melanin ; ' b,' epithelial cell mass with masses of melanin intracellularly ; and ' c,' ditto with few melanin granules (low power).

PLATE VIII.

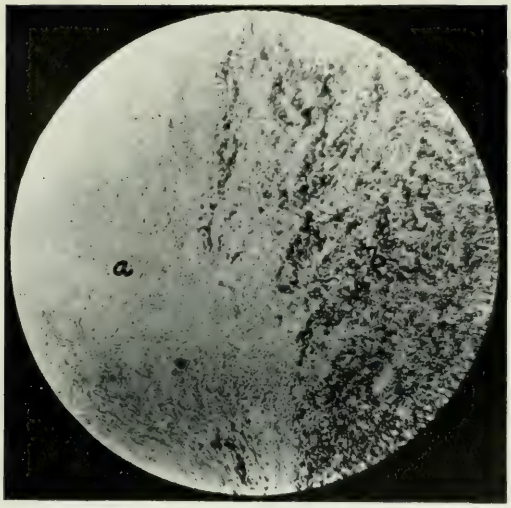

FIG. 1.

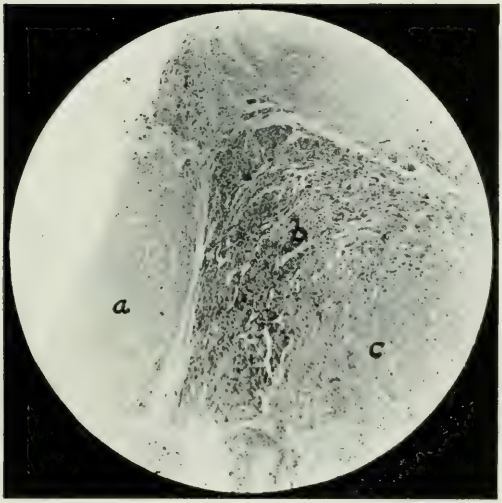

FIG. 2.

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coloured on oxidation, acts like adrenalin in raising blood pressure.

Spencer (p. 910) also states that the 'wear and tear' pigment found in heart, liver, and under the mucous membrane of the colon consists of melanin.

Having thus indicated that melanin has close relationships to adrenalin, one may now consider some of the factors that may regulate its distribution in the colouration of animals. It seems possible that the suprarenal gland may have an influence in this direction. According to Sharpey-Schafer (5), the action of adrenalin with few exceptions is that of the Sympathetic Nervous System. This nerve, amongst other things, supplies the skin structures which have pigment functions, including the pigment cells of frogs, etc. The medulla of the suprarenal, which produces adrenalin, is formed from sympathetic ganglion cells which have no peripheral axons, and adrenalin, a chemical substance circulating in the blood, produces the same effect as sympathetic stimulation and acts for it. For this reason the suprarenal has been called the emergency gland by Cannon because it musters all the resources of the body to cope with a sudden emergency just as the sympathetic does.

In the chameleon, with its changing colours, and in some fishes, the skin of which assumes complicated patterns in emergency, this is accomplished by a stimulation from a reflex from the eye, the skin, etc. Nervous shock or fright may produce the same result. In higher animals, the result may be somewhat delayed, as when the 'hair turns grey in a night.'

The similarity of action of the sympathetic and adrenalin in sudden emergency circumstances leads one to consider which will be most likely to be operative in changes of colour which take time for their production. If the sympathetic were the means active, then one would possibly have to suppose something of the nature of a muscle tetanus to be present. This seems hardly likely.

It is more probable that the change will be produced by the

adrenalin circulating constantly in small quantities.[1] Moreover, speaking of pigment cells, as the adrenalin affects the cell, and as adrenalin may possibly be transformed into melanin, this may be a means by which the adrenalin is inactivated at a spot where it will fulfil a useful purpose.

Having mentioned some general factors affecting colour distribution, the question of the effect of sex on colour as it has a bearing on pigment distribution and as it would seem to implicate more closely the suprarenal gland in the process, will now be dealt with. For the moment, the effect of the gonad on the soma will be discussed. The relation of the soma to the gonad will be treated subsequently. In order to do both, however, some points in regard to the anatomy, etc., of the ovary, especially in the fowl, will have to be discussed.

The fertilised ovum at its first division provides two cells, one of which goes to form the soma and the other the germ epithelium (Noel Paton (6)). The gonad may now be regarded as a parasite on the soma. The germ epithelium forms the sex gland and the interrenal body. The latter, later, becomes the suprarenal cortex. The relation of the parasite and the host need now to be correlated. In the case of the female, and the egg gland, this would appear to be done by the ancillary cells of the Graafian follicle and the interstitial cells of the ovary.

The ovary of the fowl of which we are more immediately speaking at present, consists of a stroma from which project egg follicles at all stages of development. Some very large and ready for extrusion consist practically of yolk surrounded by a thinned membrana granulosa. The yolk itself is secreted

[1] In this connection Redfield's work on the action of adrenalin on the melanophores of the horned toad is of interest. (Quoted by Hartmann, p. 242; see also Sharpey-Schafer, vol. ii. p. 253.)

FIG. 1.—Cock 20: inoculation with Rous tumour subcutaneously: trypan blue inoculation: local tumour: unstained: showing, 'a,' free-histiocytic cells with large trypan blue granules inside them; 'b,' fibroblastic area with fibroblasts containing few and small trypan blue granules intracellularly (low power).

FIG. 2.—Hen 165: liver: coloured hen: absorption of broken egg yolks from peritoneum: section of liver. Leishman's stain: showing large masses of a fatty material unstained inside the liver cells covered with melanin pigment (half oil immersion).

PLATE IX.

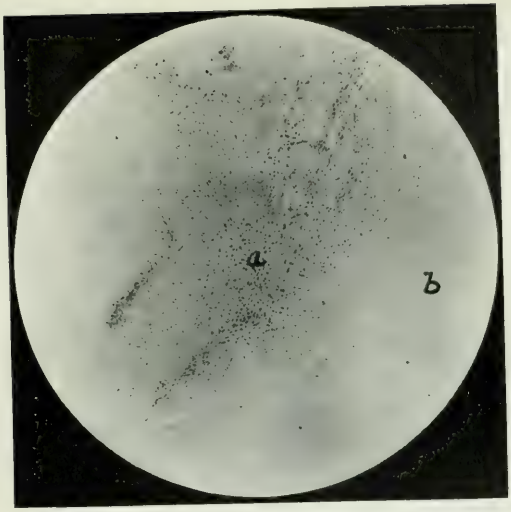

FIG. 1.

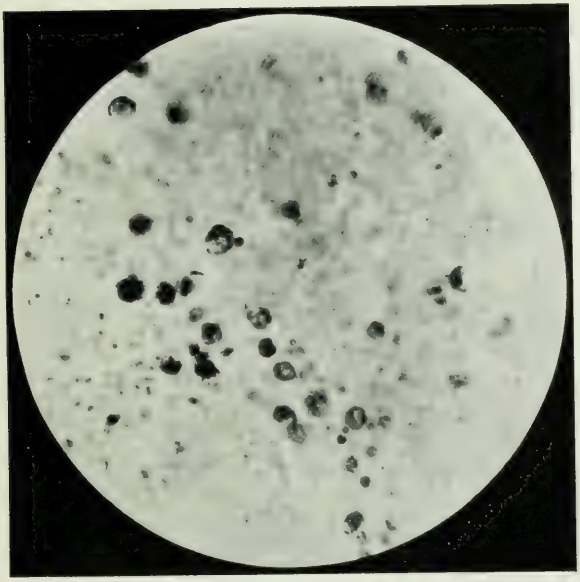

FIG. 2.

[*To face page* 50.

by the cells of the membrana granulosa and the discus proligerus. Outside the discus proligerus is the theca interna, a layer of closely packed cells. Beyond this is the loose ovarian stroma.

Pearl and Boring (7) consider that, in the fowl, corpora lutea with lutein pigment exist. The luteal cells, they state, are formed here from the theca interna, not from the membrana granulosa as in mammals. They identify the lutein pigment found by them with the lutein pigment in mammals.

Apart from the fact that no conceivable function can be fulfilled by the corpora lutea in the fowl, which voids its eggs, one has not been able to confirm their findings otherwise. In the examination of a considerable number of ovaries of fowls, pigment of any kind has rarely been found. Where it has occurred, it has been haemosiderin. Apparently Pearl and Boring did not test for this substance. They derived their luteal cells from the theca interna. This would seem to be correct so far as the origin of the cells in question is concerned. Another interpretation of their function than the one given by them, however, is possible. Examination of sections of the ovary shows large numbers of egg follicles in all stages of degeneration and disappearance. The membrana granulosa has secreted large quantities of yolk and this has to be removed. As understood here, this is done as elsewhere by large phagocytic cells, the free-histiocytes or monocytes, which wander in from the theca interna, phagocyte the yolk, and retire with it again to the theca interna and stroma of the ovary, forming thus the interstitial cells. No doubt this material is made use of again in the formation of fresh yolks.

The phagocytic nature of these cells, in the follicles, theca interna, and stroma are well brought out in fowls inoculated with trypan blue (Pl. X. Fig. 1).

Another type of cell present in the ovary of the fowl requires special mention. Great activity of the reticulo-endothelial system characterises one aspect of its activities. Numerous histioblasts and of course free-histiocytes are present. One was hardly prepared, however, to find the large

numbers of myelocytes which undoubtedly occur, especially around the small follicles. What their function there is, is doubtful. Possibly they form locally polymorpho-nuclear leucocytes which assist in the removal of detritus and take part in the physiological inflammation which occurs here on account of the great activity of the gland. At times they give rise to a myelocytoma as occurred in hen 45 (Pl. IV. Fig. 1), and myelocytic tissue as in hen No. 2 above.

The ancillary and interstitial cells bring it about that a certain number of ova arrive at maturity, being nourished at the expense of the soma. They also cause, by the agency of a hormone it is thought, certain important changes to be wrought in the soma to facilitate the working out of the destiny of the gonad. With the appliances for the fertilisation and extrusion of the ovum we are not concerned here but rather with the colouration of skin, feathers, etc., which occur as secondary sex features.

In what follows it has to be borne in mind that the cells of the suprarenal cortex are derived from the germ epithelium and that histologically they are identical with the interstitial cells of the ovary, containing a large quantity of lipoids, just as these do.

The following illustrate the effect of the ovary, testicle, and especially the suprarenal gland on epidermal sex features, especially colour. Removal of the testicle in cocks produces a neutral type of fowl, the capon, with apparent cock feathering. Removal of the ovary in the hen does the same. In both cases suprarenal cortex tissue is left and this is associated with the persistence or appearance of cock feathering in the castrated animal. Again disease of the ovary in fowls produces in them cock feathering. Presumably this may be in part from disappearance of the ovary

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Fig. 1.—Hen 191: ovary healthy hen: inoculated with trypan blue: section of ovary unstained: showing, 'a,' retrogressing large egg follicle with thickened theca interna crowded with 'pyrrhol cells' and yolk with a few 'pyrrhol cells'; 'b,' stroma with large number of 'pyrrhol cells'; 'c,' ripe egg follicle (low power).

Fig. 2.—Hen 191: Intestine: trypan blue inoculation: showing section of villus unstained: 'a,' shows deposit of fine granules of trypan blue inside the free border of the epithelium; 'b,' shows 'pyrrhol' cells out of focus in between the epithelial cells; 'c,' shows core of villus with large numbers of 'pyrrhol' cells. It would seem as if the trypan blue was changed to a leuco-compound in passing from 'c' to 'a' (high power).

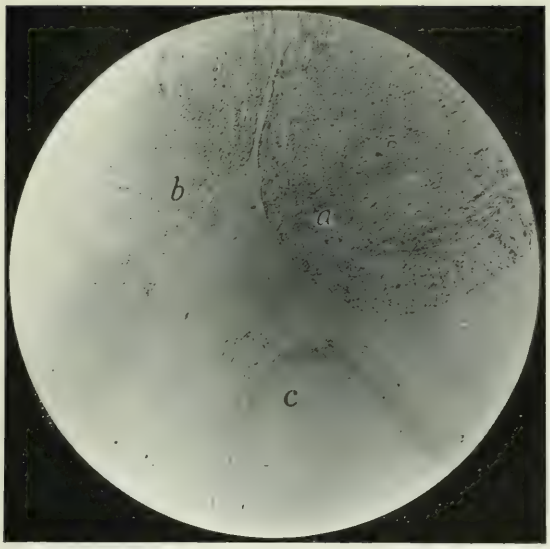

FIG. 1.

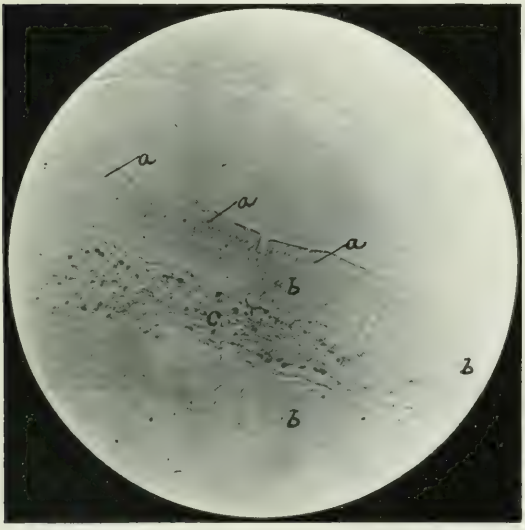

FIG. 2.

To face page 52.

as such, but there may also be an element of irritative stimulation of the suprarenal. In mammals, tumours of the suprarenal cortex in young females produces in them male epidermal characters such as hairiness, etc. (8). All these would seem to point to the suprarenal cortex being an organ for the maintenance of epidermal characters of a neutral sex, which sex incidentally, as it happens, shows features usually associated with maleness. To account for the maleness of the neutral type, Krabbe suggests that owing to the proximity of the gonads in the male to the interrenal body, some of it is incorporated in the suprarenal cortex. This seems hardly necessary. In any case, starting from this base line of neutrality, the sex gland in an individual bird could, by its presence and mass, override the suprarenal cortex, and produce the relative feathering.

It is usually admitted that these changes are brought about by hormones produced in the sex glands. The cortical cells of the suprarenals, as already mentioned, are of the same derivation and histological appearance. Presumably, they too produce their effect by means of a hormone.

The suprarenal cortex further has great influence on the development of, amongst other things, epidermal structures, as may be judged of by Elliot and Armour's studies on the anencephalous foetus (9). A reverse action may also indicate this relation. Thus Weiskotten (10) has shown that superficial burns cause great changes in the suprarenal cortex, while Cramer (11) has recorded extensive changes there also, in mice, due to exposure to cold.

It is possibly significant that, as epidermal sex characters become more pronounced in the vertebrates, the interrenal [1]

[1] It seems possible, from the authors already quoted and from Riddle's (12) observations upon gonadless birds, that the neuter conformation of the bird's body is one which shows to a slight extent the characters recognised as male when more developed. It would seem from Riddle's investigations and from those of Pézard on the castration and implantation of gonads that the inherent tendency of the germ epithelium, including the suprarenal cortex, is towards maleness. This tendency is displayed if for any reason the overriding influence of the ovary is removed. The assumption of male characters in connection with tumours of the suprarenal cortex has already been discussed.

It seems as if survival of the *type*, as represented by the germ epithelium,

body gradually envelops the chromaffin tissue or mingles with it. Possibly this may have to do with a facilitation of the play of the sex glands on the epidermal features of the animal *via* the sympathetic or adrenal medulla, which would appear to have considerable influence in this direction. That a nervous or sympathetic affection takes place in connection with sex colouring appears to be shown by Marshall's experiments on Sebright bantams. Here on removal of one testicle, cock-feathering appeared on that side first, to spread later to the other (Sharpey-Schafer). (Sebright bantam cocks are hen-feathered normally.)

This question of the relation of the gonad to the soma has been undertaken at length to show how, in a special set of circumstances, and in what manner the suprarenal may possibly affect the *distribution* of pigmentation in the skin, etc. Meirowsky, too, in his pigment studies regards the suprarenal as having a regulatory influence on the pigment exchange in the skin (Dawson, p. 672). The nearness of the suprarenal cortex to the chromaffin tissue and the chemical similarity of melanin and adrenalin might suggest a part to be played by the suprarenal medulla in the production of melanin. No evidence has been adduced that this is really the case. Dawson has discussed (p. 672) the allied question of the production of primary melanomata in the suprarenal. Tuczek, he points out, has described bilateral primary melanomata of the suprarenal. These, however, originate from the ganglion cells of the medulla and are analogous to the melanomata of other internal organs provided with sympathetic nerves. With regard to melanomata of the cortex, Tuczek and also Lucksch find that they arise from wandering chromatophores, not from specific suprarenal cells. Primary melanomata of the suprarenal

is the important thing in biology. The interrenal part of the germ epithelium, which alone is present in the gonadless birds, would appear to compel the soma of the individual to conform to type characteristics of body conformation. Sex characters would seem to be superimposed on this, once the sex gland has developed. Presumably the influence is conveyed *via* the suprarenal cortex. In addition, as the gonadless birds are hybrids, this would seem to be another effort on the part of Nature to prevent the type from being departed from by obviating their reproduction.

gland are in fact rare and usually of small size, and consideration of this whole question gives no support to the view that melanomata elsewhere are secondary to lesions, tumour or otherwise, of the suprarenal, or that the suprarenal forms melanin.

In Addison's disease, again, pigmentation of the skin is associated with disease of the suprarenals. The explanation usually given is that the diseased suprarenal, being unable to convert the common mother substance of adrenalin and melanin into adrenalin, this excess collects in the cells of the epidermis and is formed into melanin. Quite as possible an explanation is that the controlling action of the cortex, with regard to many vital functions including pigment *distribution,* has been upset, especially as it is recognised that it is not the upsetting of the medulla with regard to its adrenalin function that constitutes the gravity of this condition.

If we return now to a consideration of the nature and mode of genesis of melanomata, Dawson's conclusions (p. 687) may be quoted as an introduction. He claims that, whether the end result is a tumour morphologically resembling a melano-carcinoma, sarcoma, endo- perithelioma, or a -fibrosarcoma, the genetic process is first recognisable in the lower layers of the epidermis or in the cells of the rete epithelial processes. The tumour cells in the metastases have in his view likewise a single origin.

He mentions that it is further necessary to add that, in any melanotic tumour primary or secondary, there are present two types of cells that contain melanin pigment, the first, the true tumour cells which are producing the pigment and reproducing the cells that do so and, the second, the phagocytic cells of connective tissue or endothelial origin which ingest the pigment left by the melanoblast as it undergoes cytolysis. These latter are melanophores: they may be branching but are more usually rounded cells filled with large granules or globules of melanin, and are found in the periphery of the alveolar groups or between the strands of spindle-shaped cells. These cells may readily be confused

with tumour cells, but they are obviously of a different nature.

In reference to the same points, Ewing (13) (p. 872) comments as follows: 'The evidence accumulating in recent years from the comparative study of the physiology of the colour function in the animal kingdom is a very formidable argument in favour of the specific mesoblastic nature of the chromatophore. It is much less cogent evidence that all melanomas are derived from mesoblastic chromatophores. Theoretical considerations favour the origin of all melanomata from the mesoblastic chromatophore, while the histology of human tumours favours the origin from epithelial cells which have taken on pigmentary functions.' Spencer (p. 907) regards the epidermal origin of melanin and of the pigmented cell and the transference of such cells into the dermis as proved. The action of the non-cancerous mesoblastic cell is limited to the taking in of melanin pigment by the cells acting as phagocytes. When melanotic cancer starts, however, the mesoblastic cells take part in it and sarcoma arises. In such cases the 'dopa' reaction is shown not only by the epithelial cells but also by the connective tissue cells.

In the five cases of malignant disease of the ovaries in fowls, the three melanotic tumours occurred in coloured fowls, the non-pigmented tumours in albinos. Of the melanotic tumours, two were sarcomata of typical Rous type without any epithelial admixture, while the other was an epithelial tumour of the Graafian follicles without any admixture of sarcoma. The tumours in the albino fowls were typical Rous sarcoma, again without epithelial admixture.

With regard to the distribution of the pigment in the sarcomata, this repeated exactly the findings in the tumours of the Rous sarcoma fowls where during life the animals were inoculated with trypan blue. The large round monocytic and free-histiocytic cells were crammed with large globules of melanin, while the fibroblasts showed only a few small granules. This would seem to be an argument against the

pigment being regarded as the cause of the development of the malignancy, as has been done, for the Rous tumours when *fully developed* showed the same histological appearances on the injection of trypan blue as the melanotic tumours. In the epithelial melanoma the melanin was confined to the epithelial cells. There would seem to occur, therefore, in the ovary of the bird two distinct types of melanoma, an epithelial one and a sarcomatous one.

As it may give an indication of the mode of production of melanin in general, the question now arises for discussion as to the source of the melanin in these ovarian cases. Guthrie's experiments (14) may be of interest in this connection. He transplanted the ovary of a white leghorn into a black leghorn from which the ovary had been removed. The hen was then mated with a white leghorn cock. The majority of the resultant chickens were found to be spotted.

The fowls, in which the melanotic tumours discussed above occurred, were pigmented fowls and as such would presumably have to make provision in the egg, possibly in the yolk, for a store of chromogen to be utilised in giving colour to the chicken when it emerges from the egg.[1] Dyson (15) Kreibich (16), Ciaccio (17), have recognised melanin as being derived in some way from lipo-proteid complexes—the chromogen of melanin, belonging to the aromatic group of the protein molecule, joining up with the lipoid. Hueck (*loc. cit.*) also emphasises the association of body pigments with lipoids.

In a coloured fowl, hen 165, which had been the subject of burst egg yolks in peritoneal cavity, there was found, in sections of the liver, kidney and spleen, large fat globules in the cells, containing masses of melanin pigment (Pl. IX. Fig. 2). Hamilton (18) describes a condition of melanotic atrophy of the ovary in young pheasants which was accompanied by the assumption by the birds of male plumage. The blackening was probably a true melanosis. The yolk

[1] In this connection it is interesting to note that the chickens obtained from the mating of a coloured cock with white leghorn hens are white when hatched. Bateson, *Mendel's Principles*, 1913, p. 102.

of the eggs of black leghorns, which may be assumed to contain melanogen, when incubated with a ferment prepared by alcoholic washing of potato pulp according to the method described by Onslow (19), gave after some time a slight sooty grey deposit which did not appear when the yolk from the eggs of white leghorns was so treated.

The evidence, therefore, would seem to point to a melanogen being secreted into the yolk. As the yolk is secreted by the cells of the membrana granulosa, the appearance of melanin in tumours of this membrane, as in hen 204, can be appreciated. The yolk, however, in the degenerating atretic follicles, is removed by the phagocytic cells of the ovarian stroma or theca interna—the monocytes or free-histiocytes. Such cells return to their place of origin filled up with yolk material. It is easy, therefore, to imagine that they could produce melanin on their becoming sarcomatous, especially if Spencer's view is correct that melano-sarcomatous cells contain the ' dopa ' oxydase. In any case, in the present instance, the sarcoma cells must have produced the melanin for there was no concomitant epithelial melanoma present from which they could obtain it.

Attention has been drawn to the fact that the reaction of the melanin to the tumour cells in these myxo-sarcomata was exactly duplicated by the relation of trypan blue to the same cells. An observation in one of Goldmann's early papers (27) on the subject of *intra vitam* staining is important in this connection. He states (p. 150) as follows : ' cell plasma which accepts fat and lipoid stains such as sudan, scarlet red, and nile blue, also shows a marked tendency towards vital stains. By examination of the " pyrrhol cell " in its transformation into the spindle cell of scar tissue and during the phases of disintegration under the influence of bacillary necrosis the " pyrrhol cell " accepts the vital stain only. Whereas, during the above changes, its affinity for the vital stain decreases, that for fat stain increases. Hence he is inclined to assume that under normal conditions fat or lipoid substances of the cell plasma unite with proteins and form loose compounds liable to *intra vitam* staining.

Once this coherence is broken the histo-chemical fat reaction becomes evident whereas the vital stain is lost. . . . That the vital stain is not directly due to the presence of a fat or lipoid bodies may also be inferred from the fact that he had hitherto failed to discover a fat or lipoid solvent for any of our vital stains.'

The significance of this statement for the subject in hand would appear to be considerable. It would seem to indicate that the ' pyrrhol cells ' carry about with them—differing in this way from other cells—large quantities of a lipo-protein complex.[1] We have seen that several authors have associated the occurrence of melanin with a similar complex, while, in the observations above described in regard to tumours of the ovaries of fowls, it would appear that melanogen, becoming melanin in the tumour cell, is associated with the lipo-proteins of the egg yolk. Melanin formation would seem, therefore, to centre round the ' pyrrhol ' cell or free-histiocyte as the carrier of a chromogen. In the epidermal structures it seems likely that this is passed on to the epithelial cells and there is transformed into the melanin of the skin and hairs and is eventually got rid of. Cancerous changes may of course arise in this epithelium and produce melano-carcinoma. It seems, however, that sarcomata might arise also. Thus, Jäger (21) describes the origination of melanotic sarcoma in the patches around the tail, anus, prepuce, etc., which retain their dark colour in grey horses, the hair of which becomes pure white with age. In such cases, the pigment metabolism must become abnormal in these limited areas, owing to the whole of the rest of the skin ceasing its melanin producing and excreting function. In such cases, histological evidence, according to Jäger, shows at first a simple pigmentation of the *cutis vera*, which then becomes sarcomatous and attacks the subcutis. The

[1] The behaviour of the monocytes in lipoidaemic diabetes, in Gaucher's disease, as also in Niemann's (21) splenomegaly, may be cited in this connection. Anitschkow (22) has noted the occurrence of great accumulations of fat in the macrophages of the spleen in animals fed on a diet rich in cholesterol. He identified the type of cell by means of trypan blue injections.

epithelium is not affected. Similar conditions, brought about in other ways however, could be postulated for the origination of carcinomatous and sarcomatous tumours of the ovary of the fowl.

One can only hazard a guess at the seat of origin of the chromogenic lipo-protein complex. It seems likely that it will arise in some central organ devoted to the building up of lipo-proteid material, possibly the liver. Its distribution possibly by the ' pyrrhol cell,' as we have seen earlier, seems to be under the control of the sympathetic and the suprarenal with its cortex and medulla. The latter would appear to function specially in sex colouring.

Melanin seems to be got rid of largely in the epidermis by the falling off of scales and hairs. Other modes of excretion, however, seem probable, such as by the kidney, the liver, and the intestinal epithelium. The excretion of melanin or melanogen seems to be duplicated by that of trypan blue.[1] Thus melanin or its precursor is excreted in certain conditions in the urine, producing melanuria. This is paralleled in trypan blue inoculation by the deep blue staining of the secreting tubules of the kidney. Again, there accumulates in large quantities in the intestinal mucosa both in the lower animals and man a black pigment. Spencer (*loc. cit.*) states it to be melanin. Heuck (*loc. cit.*), however, says it is more nearly related to lipo-fuscin than melanin. In trypan blue inoculation, the whole intestinal tract from the duodenum down is coloured intensely blue. When this tissue is examined histologically, the central core of the villi is found to be crowded with ' pyrrhol cells.' Some of these are seen to be escaping through between the epithelial cells while the surface layer of the cytoplasm of the epithelial cells themselves is crowded with minute blue granules. Excretion of excess iron pigment would seem to take place by the same route. Fig. 1, Plate XI, shows the

[1] Cases have been recorded of general melanosis in the fowl which exactly repeat the appearances found in fowls inoculated with trypan blue. No local lesion is noted. (Ward and Gallagher, *Diseases of Domesticated Birds*, p. 272.)

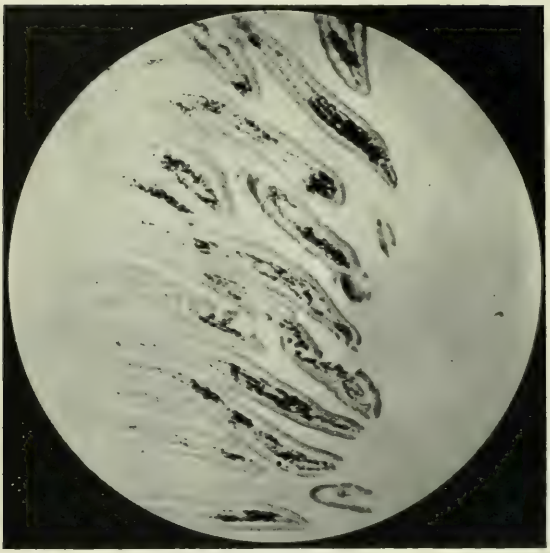

FIG. 1.

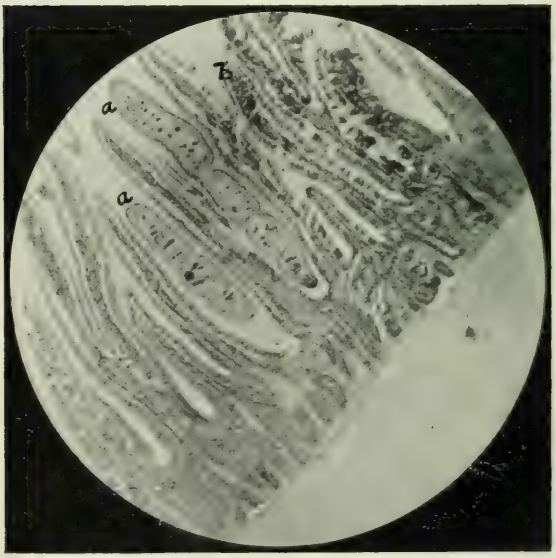

FIG. 2.

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large quantities of iron pigment that may occur in the intestinal villi in leucosis of fowls. Although tape worms are present in large numbers (Fig. 2), the iron pigment is not due to local haemorrhages as these are not present. Judging from the histological appearances found in the liver in trypan blue inoculation, the pigment would seem to be excreted by the bile. In addition to the Kupffer cells being full of it, the liver cells themselves contain large numbers of small granules of the dye. Whether melanin is excreted also by this channel does not seem to have been determined.

In the discussion of the anatomy of functions of the ovary and suprarenals, the works of several authorities have been laid under contribution without acknowledgment at the time. Owing to the shifting scenes of the argument it was thought that the introduction of numbers at every reference to keep pace with it would load up the text unduly. Among the authors drawn upon are the following : Sharpey-Schafer (*loc. cit.*), Swale Vincent (22), Cowdry (23), Noel Paton (*loc. cit.*) (24), Mason (25), Weil (26), Cannon (27), Quinby (28), Bayliss (29).

SUMMARY

Melanotic sarcomata and a melanotic carcinoma of the ovary of the fowl are described and discussed. This indicates that in the fowl at least, two types of melanomata—mesoblastic and epithelial—occur.

It is suggested that the melanogen which gives rise to the melanin in these cases, is derived from a melanogenic lipo-protein complex secreted into the egg yolk as a provision for the colouring of the young chick.

It is also suggested that melanin metabolism is centred round the cell which takes the dye in intravital staining—the ' pyrrhol cell.'

FIG. 1.—Hen 176 : duodenum : leucosis : H.Cl. and Ferrocyanide of potash : showing large quantity of iron pigment in the core of the villi, and also just inside the outer border of the epithelial cells : note the iron pigment occurs only at the tips of the villi (low power).

FIG. 2.—Hen 176 : duodenum : leucosis. Leishman's stain : showing tape worms (Davainea pro-glottina) among the villi : ' a,' tape worms, ' b,' iron pigment at tips of villi (low power).

No certain source of *origin* of the melanogenic lipo-protein complex can be suggested. It does not appear to be produced in the suprarenal gland. It is possibly formed in the organ which is usually regarded as having specially to do with the elaboration of fat products—the liver.

With regard to its *distribution*, it would seem that the sympathetic and the suprarenal gland—cortex and medulla —play an important rôle. In its *excretion*, its behaviour seems to run parallel to that of trypan blue and iron pigment in haemosiderotic conditions. Indeed the three in their association with fatty substances in the cells and organs in which they occur and in their excretion conduct themselves in exactly the same way.

VII

ON THE RÔLE OF THE LYMPHOCYTE IN AVIAN PATHOLOGY

As has already been stated no case of lymphatic leucosis has been met with in the series of cases on which this investigation and a previous one on leucosis (1) are based. Cases were naturally expected owing to Ellermann's (2) mention of their frequent occurrence. One can find as yet no adequate reason for their non-occurrence. It may be that the number of cases investigated is not large enough. This can hardly be so, for Ellermann alleged that he had met with them in a much smaller series. Possibly it is related to the fact that in fowls lymphatic glands and some other lymphatic structures are absent.

In fowls, great difficulty surrounds the question of determining when and if one has to do with lymphocytic tumours. Ellermann sensed this (*loc. cit.* p. 44) and formulated rules for guidance. He recognised the difficulty of determining the nature of such cells by morphology alone or this combined with bio-chemical reaction and finally gave it as his opinion that a consideration of the histogenesis in each individual case was the only sure method of establishing the nature of a cell.

A great part of the ambiguity in connection with the lymphocyte would appear to be related to the terminology in use. Maximow (3) demonstrated the importance of the lymphocytic-wandering-cell of the embryonic tissues. By some means this cell, although differing even morphologically from the lymphocyte, came to be called the 'large lympho-

cyte of the embryo' or shorter the 'large lymphocyte,' and the term was then applied to cells of the fully developed animal. Thus, the large lymphocyte of adult lymphatic glands came to be identified not only morphologically but also physiologically and potentially with the embryonic lymphocytic-wandering-cell. From this arose one variety of the monophyletic doctrine of blood cell genesis. This subject has been discussed elsewhere (McGowan, *loc. cit.*) and need not be gone further into here. It will be sufficient to say that Maximow in later publications has stated that a better term for the lymphocytic-wandering-cell than large lymphocyte would be haemocytoblast. A reference to the diagram of blood cell formation will show that criticism could be applied even to this term—the term haemohistioblast being apparently more appropriate.

The fundamental error involved in this question would seem to be the assigning an identical physiological function and development to cells which for the time being have an apparently similar morphology. A glance at the diagram of blood cell formation (p. 2) will possibly make clear what is involved here. Thus, in the first instance the haemohistioblast, the haemocytoblast, and the histioblast have identical morphology, but the two latter have quite different potentialities. The same may be said of the various plasma cells as also of the lymphoblast, myeloblast, and monoblast, at least if examined in sections.

The important point to be noted, therefore, is that one is unable to predict in cells of apparently identical morphology what their eventual fate will be. One needs to know their past history, their present surroundings, and indeed, as matters are, some of their future history as found in further developmental stages of cells exactly like them. It is possible that, at a certain stage of the journey, all the roads of development open to cells of this morphology may be possible for an individual cell. It is questionable, however, whether, in the majority of cases, once a cell has been committed to a certain route—conditioned and governed as it is by environment—it can turn back and go along a different path.

While it may be possible, in certain cases in blood films, to differentiate by morphology, staining methods, and biochemical reactions (oxydase reaction) such ' lymphoid ' cells as the myeloblast, lymphoblast and monocyte, it is very difficult to do so in sections made from fixed tissues. In the latter case recourse has to be had to other measures. Leaving out of account for the present tumours based on the monocyte or free-histiocyte, the main problem is one of determining whether a certain tumour is myeloblastic or lymphoblastic in nature. In the case of a myeloblastic tumour one would expect to find, either in the tumour itself or in some of its concomitant lesions, myelocytes in some stage of formation. Again one would expect the emphasis of the lesions to be laid on the myeloid organs, such as the bone marrow, while the lymphocytic organs would be unaffected. Conversely, in the case of a genuine lymphocytic tumour, one would expect the weight of the disease to fall on such lymphocytic organs as the masses of lymphocytic tissue lining the alimentary tract (there being no lymphatic glands in the fowl), the thymus, and the malpighian bodies in the spleen, while one would anticipate that the marrow, a myeloid organ, would if affected at all show a lymphocytic transformation. The periportal tissue of the liver in this scheme of differentiation occupies a neutral position. One may suppose from the existence of an artery and a vein there, a subject to be discussed later, that either the myeloid or the lymphocytic type of tumour may develop and leave it to the actual finding or not of myelocytes to decide its nature.

With this scheme of differentiation outlined, the following may be submitted. They have been derived from a minute examination, naked eye and microscopic, of over 150 cases of fowls with leucosis, leucotic and mesenchymal tumours. In no case has there ever been any hypertrophy or tumour of the lymphatic tissue of the intestinal tract. The thymus, examined in every case, was found hypertrophied in only one. This was a case of Rous tumour and the lesion was one of the tumour. In every case the marrow was found pro-

foundly affected, being in a state either of histioblastic, myeloblastic, or myelocytic hyperplasia. There was never any condition present in this tissue which could even remotely be interpreted as lymphoblastic. With regard to the ' malpighian bodies ' of the spleen, there was never any lymphocytic enlargement. Enlargements occurred in Rous tumour cases but the cell elements there were of the tumour type. The spleen pulp was often greatly enlarged. This was due to the accumulation of myeloid cells, or in some cases—intravascular leucosis—of haemocytoblasts. In the liver, where for the sake of argument an equal possibility of myeloid or lymphatic tissue developing in the periportal spaces has been supposed, the proliferation in every case, where it was not of the Rous-sarcoma type, was myeloid in nature.

In regard to tumours formed of more embryonic cells, such as the plasma cell and the histioblast, the same difficulty of assigning a myeloid or lymphocytic lineage was encountered and was met by an application of the same canons of differentiating. By this means, it was established that tumours of these types also belonged to the myeloid lineage.

So far one has considered the lymphatic system in the fowl in a limited way with reference to differentiation of its possible tumours from those with a myeloid origin. It will now be necessary to discuss it in a more general fashion with a view to dealing later with some further points in reference to the types of tumour treated of here.

The lymphatic system as a whole is derived by an evagination from the venous side of the circulation. It is a closed system towards the tissues, projecting into them like the fingers of a glove. It serves to gather up material from the tissues which has exuded into them from the closed circuit of the blood, from the absorbent surface of the intestines, and in certain instances, as in the case of injuries, etc., of the skin, from the epidermal surface. In all these cases, waste, metabolic products from the tissues or foreign material from the outside, gains access to the lymphatics and is in process of obtaining entrance to the blood stream. It is

obvious that some sifting arrangement on the course of the lymphatics would be an advantage—for screening out particulate matter and for altering foreign material before its entrance into the blood.

Lymphatic glands on the course of the vessels, lymphatic tissue along the intestinal tract, and even ' small celled infiltration ' round an irritant [1] in the tissues would seem to be of this nature.

With regard to the lymphatic glands, they appear round the lymphatic vessels late in embryonic life. They would seem to be derived from the perivascular sheaths of the arteries (*vide* Jolly (4), p. 729) which bulge into the lumen of the lymphatic vessels, much as the liver and the splenic artery bulge into the portal vein. This will be discussed later.

The lymph follicles, germ centres, and the lymphatic cords would appear, therefore, to be a hypertrophy in a lymphatic direction of the reticular elements in the perivascular sheath of the artery. The original lumen of the lymphatic is much chambered and broken up and is lined by an endothelium having much of the character of that lining the sinusoids of the liver and the splenic sinuses—that is to say, it is discontinuous.

There is an afferent and an efferent lymphatic and the course of the lymph is from the one to the other through the sinuses. From the nature of the gland, with its intricate meshwork of lymph channels lined with a discontinuous endothelium with phagocytic properties, its efficiency as a biological filter will be evident. In the performance of this function it may be that the lymphatic tissue subserves in part a purely mechanical function of giving body and support to the filter. In addition, however, the gland supplies lymphocytes to the blood. It may be supposed that these gain entrance to the lymph channels through the breaks in

[1] See in this connection Dominici and Rubens-Duval quoted by Jolly, *loc. cit.* p. 581. Conversely, it is possible to regard the occurrences in normal lymph glands as being of the nature of a chronic physiological inflammation.

the endothelium. The usual view is that this occurs by means of amoeboid movement. It should not be overlooked, however, especially since this amoeboid capacity on the part of the lymphocyte is a much debated question, that here (as in the lymphatic tissue of the intestine, subject to the peristaltic action of the muscle wall) the lymphatic glands, owing to movements of the muscles of the body, etc., are being constantly massaged. Hence lymphocytes, without any effort on their part, may be squeezed out into the lymphatics and from there sucked or massaged into the venous circulation. The possible fate and function of these lymphocytes will be discussed later.

In the intestinal lymphatic structures the intestinal epithelium is infiltrated by leucocytes. The lymph nodules correspond to the cortex of the lymph glands. Here, again, a lymphatic sieve is placed across the entrance of foreign material into the blood. There is a slight modification in this instance as compared with the ordinary lymphatic gland. Efferent lymphatics, closed at their end, lead from the cortex towards the lymphatic trunks. Through the epithelium, infiltrated with leucocytes, the afferent stream carrying foreign material travels to the cortex. There may also be, however, an efferent stream from the cortex through the epithelium to the intestinal surface. The infiltrating lymphocytes may be evidence [1] of this. In any case Bunting (5) has drawn attention to the enormous loss of leucocytes from the blood that occurs to the intestine. The findings in the intestinal mucosa in the trypan blue experiments may be noted also in this connection.

It would seem possible, therefore, that here may be the ultimate fate of a large part of the leucocytes of the blood and especially of the lymphocytes. In the lymphatic glands, the lymphocytes, in their capacity of a biological filter, have been dealing with possible noxious material. In this process they may have been injured or, at least, their capacity for

[1] Jolly, however, regards the combined structure as a special type of gland—lympho-epithelial glands. The thymus and Bursa Fabricius are other examples.

carrying on their function may have been affected. They may be massaged out into the lymph stream and from there gain entrance to the blood vessels. No definite function in the blood stream has ever with adequate reason been assigned them. On the other hand, it is a feasible suggestion, especially having regard to Bunting's results and the appearances in the intestine, that they are simply travelling *via* the blood channels to the intestinal surface to be finally extruded there.

The next question to be discussed is the formation of foci of ' small round cells ' around a chronic not too severe irritant in the tissues. This accumulation is, by many, attributed to a diapedesis from the surrounding blood capillaries. The idea is based on the presence of lymphocytes, caught in the act of passing through the walls of the capillaries, and on the absence of local mitotic figures—indicating local multiplication. With regard to the former, it is difficult to see how the decision is arrived at that the lymphocyte in question is actually passing *out* of the vessel, while, with regard to the latter, it has been stated by some observers (*vide* Maximow (6), p. 553) that such multiplication here occurs by a process of amitotic division, whether in the lymphocyte as such or not is not quite clear. In any case, the absence of mitotic figures in such collections of cells seems quite definite and could be explained on the following grounds. In such lesions—as in tubercle foci—reticular cells are notably present. These could form histioblasts which, by dividing amitotically, could produce large numbers of similar cells, which could further ripen into lymphocytes. This is the process which would seem to take place to a great extent in the ' germ centres ' of lymphatic glands as will be discussed later. Indeed the whole process could be regarded as the local construction for entirely local purposes of a simplified lymphatic gland. It would be thrown across the path of entrance to the blood of noxious material.

The periportal tissues of the liver have now to be discussed. The liver, with its duct and accompanying hepatic artery, forms an invagination into the portal vein. The hepatic

artery, after supplying the liver cells, eventually debouches into the subdivisions of the portal vein—the sinusoids. The endothelial lining of these portal vein spaces is discontinuous and beneath it is a subendothelial tissue having in the embryo of some animals—not the fowl, however—haemotopoietic functions.

The spleen is formed on the same principle. Here, however, there is no invaginating organ. The splenic artery (with corresponding vein) invaginates the portal vein. There is a great lymphatic development of its perivascular sheath in some animals—not the fowl, however—which fuses with the subendothelial tissue of the portal vein which in its turn is lined by a discontinuous endothelium. The blood of the splenic artery passes through the malpighian artery with its lymphoid sheath and pours itself *via* the ellipsoids or artérioles à housse which constitute the greater part of the so-called malpighian bodies of the spleen of the fowl [1] either directly into the splenic sinuses, or into the subendothelial tissue first, from whence it finds its way into the splenic sinuses *via* the discontinuous endothelium.

The following special points may be noted in the construction of the liver and spleen. In the liver the main functioning parts are the endothelial tissues together, of course, with the liver cells. The hepatic artery in ordinary circumstances does not appear to do more than supply blood to the liver cells. The endothelial and subendothelial cells—formed in reference to a *vein*—produce red blood cells, myelocytic cells and monocytes for blood circulation, at least in the embryo and in certain animals. In the spleen, the functioning portions are the endothelial and subendothelial cells of the portal vein and the perivascular tissues of the splenic artery. Again, the endothelial and subendothelial tissues—formed in reference to a *vein*—can produce in the embryo and in certain animals red blood cells, myelocytic and monocytic cells for blood circulation, while the perivascular tissues of the splenic artery produce lymphocytes and free-histiocytes —the latter with a tendency to become fibroblasts.

[1] *Vide* Jolly, *loc. cit.* p. 767.

It should be noted, therefore, how perivascularity is associated with the formation of lymphocytic cells, while endothelial and subendothelial situation is associated with the formation of myeloid types. This would seem to hold even in lymphatic glands. The lymphatic vessel is, in origin, a portion of a vein, and its endothelial and subendothelial tissues can give rise to myeloid cells such as red blood cells, myelocytes, etc., while the perivascular sheath of the artery gives rise to the lymphocytes. The monocyte and free-histiocyte may arise in either site. There may, however, be justification in the above separation of site of origin for Sabin's (7) differentiation of the cells arising from the two sites, by means of the supra-vital technique, into monocytes derived from the primitive reticular cells and clasmatocytes from endothelium.

Such a scheme would appear to explain the appearance in disease conditions of haematogenic tissues of various types in the liver and spleen.[1] In this connection, the lymphocyte potentialities of the perivascular tissue of the hepatic artery [2] should not be forgotten, especially in relation to lymphatic leucaemia conditions in man, nor should the myeloid potentialities of the endothelial and subendothelial tissues of the lymphatics be lost sight of in connection with the development of myeloid tissue in the lymphatic glands.

These findings have now to be applied to the consideration of some of the other points raised in connection with the present problem. In the embryo, at a time when, as yet, the mesoblast is not encroached on by organs coming from the epiderm or endoderm, its structure, so far as it has one, is oriented round the various blood vessels ramifying through it. The mesenchymatous tissue is, thus, in its early days associated intimately with the vessels, the vessel walls to enclose the blood constituting the first formed mesenchymatous structure. This circumstance possibly explains the great importance of the perivascular tissues as a source of mesenchymatous elements (reticular elements) later on.

[1] In the kidney also: for intravascular erythro-poiesis, etc. in the kidney see Naegeli, pp. 131, 132.

[2] *Vide* Naegeli, p. 426: for same condition in bone marrow, p. 425.

Already it has been shown that the spread of the Rous tumour, in the region at some little distance from the body of the tumour, occurs by means of a perivascular growth. The cells in this growth range from histiocytes or reticular elements through histioblasts, free-histiocytes to fibroblasts. ' Small round cells ' are present in many cases also. This raises again the question of whether morphology is a sufficient criterion for judging of the nature of a cell. By many the ' small round cell ' in this situation is regarded as a lymphocyte which has migrated out of the blood to this situation. For reasons already discussed, it is more likely in the tumours to be in the majority of cases a small free-histiocyte which has been formed locally. Even in cases unconnected with such tumour growths, as in chronic inflammatory conditions, it has been too readily assumed that the collection of small round cells are lymphocytes which have emigrated from blood vessels. They may be, of course, lymphocytes which have been formed locally: on the other hand, they may not be lymphocytes but small free-histiocytes as already mentioned.

Tumours of the Rous type would, therefore, appear to arise from the portion of the reticulo-endothelial system—the fixed histiocytes or reticular cells—which surround arteries. These cells develop along a lineage ending in fibroblasts. Owing to the fundamental nature of these cells, however, they can hardly be stirred up without other lineages being set off on a wayward career. Thus may be explained the hyperplasias of the myelocytic lineages in the marrow, periportal spaces of liver, kidney, etc. There would seem, however, to be a disinclination in the fowl to develop, under any condition of stimulation (the correct stimulus may of course be absent) of the reticulo-endothelial system or otherwise, tumours of the lymphatic lineage.

A word may be said, in conclusion, regarding the nature of the ' germ centres ' in lymphatic tissue. For a long time it was considered that the ' germ centres ' gave origin to the lymphocytes by a process of mitotic division, an opinion still adhered to by Jolly (*loc. cit.* p. 697); Latta (8), owing

to the absence of mitotic figures in them and for other reasons, considers them to be areas of degeneration due to lack of blood supply. In the process of degeneration, he thinks, they assume phagocytic powers, engulfing degenerating small lymphocytes. Mottram (9), also, does not regard the germ centres as containing the mother cells of the lymphocytes. He notes what he interprets as phagocytosis of lymphocytes by the reticular cells of the germ centres. He suggests (p. 476) also that the cells of the follicles are changed into plasmoidocytes—a form of plasma cell—and that these plasmoidocytes by mitotic division produce lymphocytes. He notes that the 'germ centres' are arranged round central blood vessels. Aschoff (10) states that, whereas the germinal cells were previously regarded as the sites of lymphocytic production, it has recently been contended especially by Hellmann (11), that cellular proliferation in these areas is the manifestation of defence reactions against various toxic infectious agencies. From his own experience of the lymph node alterations accompanying appendicitis, he considers that there is no doubt that every injury to the lymphocytic elements, however slight, initiates a large cell proliferation of the reticulum which leads to extensive phagocytosis of the degenerated lymphocytes.

A harmonisation of these various views seems possible as follows: The perivascular sheath of the arteries in the 'germ centres' contain large numbers of reticular cells with multiple potencies. These can produce histioblasts which multiply by amitotic [1] division. From these histioblasts, *via* the plasma cell, can be formed on the one hand lymphocytes and on the other, free-histiocytes or phagocytic cells, a combination which would give rise to the appearances observed by Latta, Mottram, etc. It is well known that lymphatic tissues are very mobile and actively changing (Ewing (12), Jolly, *loc. cit.* pp. 756, 740). Their reactions to hunger and X-rays (*vide* Jolly, p. 862) demonstrate this and there are all intermediate stages of waxing and waning. The waxing phase will be seen histologically in the

[1] *Vide* Jolly, *loc. cit.* p. 581, for amitotic division in 'germ centres.'

mitotic figures in the plasma cells as referred to by Mottram (*loc. cit.*).

This view would appear to be confirmed from the appearances observed in the spleens of fowls. Here, however, one has to do with the formation of monocytic rather than lymphocytic cells. At times the central artery of the malpighian lobule (ellipsoid, artériole à housse) was found to be surrounded by a zone of quiescent eosinophil reticular cells. Beyond this was a peripheral area, in contact with the pulp, consisting of histioblasts, plasma cells and phagocytic cells. Haemosiderin, in cases of anaemia, was especially present in the cells of this zone demonstrating the phagocytic activity of such cells. It was never found in the reticular cell zone. In other cases, the reticular zone was thinned down to a practical absence and the artery was more immediately surrounded by a very cellular zone. This again consisted of histioblasts, plasma cells and phagocytic cells. These cells were more or less intermixed. The angular histioblasts with their deep-blue cytoplasm and their faintly staining nucleus could easily, however, be recognised. In another group of cases the cells of the reticular zone were swollen out and their outlines thickened while fairly large masses of basophile material were to be found in the almost unstained cytoplasm, recalling the appearances described in lymphatic ' germ centres ' by Latta, Mottram, etc. Morphologically, these cells were identical with the cells present in the plasma celled tumours, such as in hen 158 (*see* Pl. IV. Fig. 2). This appearance of the reticular zone (often found present in normal spleens in a slight degree) was exaggerated in disease conditions, as it was also in the round collections of cells found lying sometimes adjacent to the malpighian artery in normal young fowls—the ' secondary follicles ' [1] of Jolly (Pl. V. Fig. 1).

As these basophile bodies in the cytoplasm of the cells

[1] For a discussion of ' germ centres ' or ' clear centres ' and of ' secondary follicles ' or ' nodular germ centres,' see Jolly, pp. 600, 699, 756. The ' secondary follicles,' according to Jolly, do not consist of reticular cells but of lymphoid cells. Both, however, as just noted, showed the plasma cell change in disease conditions.

occur very largely in plasma celled tumours of the liver, kidney, and elsewhere (*see* Pl. IV. Fig. 2), it seems doubtful if their interpretation in the ' germ centres ' as residues of phagocyted lymphocytes is in all cases a correct one. Phagocytosis of lymphocytes is quite a possibility in this situation, but they would seem rather in most instances to be stages, it may be irregular ones, in the transference of the diffuse chromatin of the cytoplasm of the histioblast to the nucleus in the process of the ripening of this cell to the lymphoblast or the free-histiocyte as the case may be.

SUMMARY

The difficulty of distinguishing the various ' lymphoid ' cells in tumour conditions of the fowl has been emphasised and discussed.

The essentially similar structure, *qua* haematological tissues, of the liver, spleen, and lymphatic glands has been pointed out. In doing so, attention has been drawn to the circumstance that endothelial and subendothelial position in *veins* is associated with the formation of myeloid cells, while perivascular position in relation to *arteries* is associated with the appearance of lymphocytic cells. Free histiocytes, on the other hand, may arise in both situations.

The bearing of these various points on the occurrence and development of mesenchyme derived tumours in the fowl, such as Rous tumours, leucotic tumours, leucosis, etc., is discussed in the text.

Various disputed points, in connection with the anatomy and physiology of the lymphatic apparatus, are also referred to and new interpretations of certain appearances submitted. The significance of the absence of lymphatic tissue in the spleen of fowls in the interpretation of disease appearances is stressed.

ADDENDUM

Since this was written there has appeared a paper entitled ' A review of the structure and function of the spleen ' by

Tait in the B.M.J. of Aug. 20, 1927, p. 291. In surveying the subject from a comparative anatomy point of view, he points out that lymphoid tissue is not a fundamental or essential component of splenic structure. This adds support to the views advanced here regarding the appearances present in healthy and diseased spleens of fowls.

VIII

AETIOLOGY OF TUMOURS

In 1910 (1) Peyton Rous described a spindle celled sarcoma, which he had obtained as a sporadic tumour in a Plymouth rock fowl. At first, it could only be transplanted into near relatives of the fowl which originated it. After a long series of transmissions from fowl to fowl, the scope of inoculation increased. In the end it could be transmitted to most breeds of fowls, still, however, retaining its species specificity. Rous found also that the tumour could be transferred to other fowls, not only by living cells but also by dried tumour tissue and cell-free filtrates of the tumour tissue extract. This tumour—Rous sarcoma No. 1—has been cultivated in fowls since it was originally obtained and forms the basis of much of the work on cancer at the present time.

In a series of papers commencing in 1924 (2-18) Carrel has developed his views regarding its nature. In his first papers he made considerable progress with regard to the cultivation *in vitro* of the causal agent. He noted that it was very fragile in nature and that its activity disappeared in less than 24 hours in a suspension in a fluid medium such as saline solution, broth, serum, etc. Later, he found that the addition of a piece of muscle or leucocytes to a suspension of Rous tumour which had lost its activity at incubation temperature caused its powers to develop again. In subsequent papers he has further investigated and particularised the conditions of its growth. In doing so he has been able, by means of a method of soaking small discs in varying dilutions and inoculating a series of them into a single fowl, to meet the

difficulty of varying susceptibility in different fowls and to test with some degree of accuracy the potency of different cultures of the agent at different times and under different conditions. By such means he has proved not only that the agent increases in amount in cultures *in vitro* but has also been able to define some of the conditions which influence this increase. He ascertained, thus, that for increase of the agent living cells were required in the culture medium. The increase also depended on the amount of these living cells present. Any condition, such as freezing or anaerobiosis, which lessened the vitality of these cells rendered at the same time the medium less suitable. He found also that the agent decreased in cultures where the cellular elements were fibroblasts but increased where they were monocytes. He observed that culture strains of fibroblasts, obtained from Rous and other like sarcomata, very rarely produced sarcomata upon inoculation into fowls. The inoculation, however, of cultures of macrophages from the same tumours practically always determined their appearance. As a source of supply of living cells for the cultures, embryo pulp, and fragments of spleen could be used as well as monocyte cultures, and the more of these added to the cultures, the greater was the amount of the agent produced. With dead cells in the culture medium, the agent survived but did not increase in amount. Cultures of normal monocytes, when inoculated with the filtered extract of Rous tumour, often, but not always, assumed the fibroblastic appearance of cultures of Rous sarcoma. The cells, however, in the case of monocytic cultures shed off the agent into the culture fluid, a thing which did not happen when fibroblastic cultures were used. He found, also, that in such cultures the monocytes became diseased and acquired the power of digesting the fibrin of the culture medium. By additional experiments, directed solely to determine the point, he concluded that the rôle of the living cells in the cultures is to promote directly the growth of the active agent.

He embedded chicken leucocytes in small blood plasma pellicles and placed these in a tube with a fluid consisting

of a mixture of plasma, Tyrode's solution and embryonic juice. On incubation with daily replacement of the fluid, he found that two months old cultures could produce daily one cubic centimetre of a very virulent fluid.

Pursuing his investigations, he found that the injection of the embryonic juice of chickens, subcutaneously, into fowls produced a non-malignant teratoma. If, however, there was injected at the same time a small amount of tar, a weak dilution of arsenious acid or indol into the same fowl, the tumour became malignant and took on all the characters of a Rous tumour. With material from such tumours, originated as has been seen by purely chemical means, Carrel carried out all the tests, cultural and otherwise, already performed by him with Rous tumour material. He mentions only one failure as occurring. With indol sarcoma material he was unable to infect cultures of monocytes.

He concludes against the existence in Rous sarcoma of a living multiplying particulate virus, especially when, as we have just seen, results, similar to those obtained with Rous sarcoma, occurred in the sarcomata produced by tar, arsenic, and indol. As regards the nature of the agent, he suggests that Rous and the other sarcomata are really a disease of the monocytes. The agent penetrates the monocyte and without interfering with its reproductive power causes it to die prematurely. Meantime the dying cell secretes more of the Rous agent. When this is injected into a fowl, it is destroyed in a short time unless it is taken up by a tissue macrophage. This infected macrophage multiplies and infects other macrophages but eventually dies. While this is going on, the growth promoting substances contained in the macrophages are set free. They bring to the fibroblasts and other cells of the neighbouring tissues the food material and stimuli necessary for multiplication. He compares the Rous agent with the bacteriophage. The monocytes are infected by it, are subsequently destroyed, and set free from their substance a further amount of the agent. This occurs much in the way that the bacteriophage infects bacteria, subsequently lyses them, and sets free more bacteriophage.

Elsewhere, Carrel suggests a further action. In fluid cultures, monocytes are unable to form a tissue. The life of the monocytes, in a fluid culture with Rous agent, he suggests, becomes impossible. They therefore transform themselves into cells capable of living under the conditions present in the culture—that is, into fibroblasts which are not sensitive to the agent.

In the genesis of a sarcoma, Carrel thinks that there are two factors at work. Firstly, there is a focus of cells in active multiplication, and secondly, there is a toxic substance acting on these cells. He cites, as an example, the action of tar on experimentally produced teratomata in the fowl. He points out that the factor, which conditions the change of the normal cells into tumour cells, is not the same as that which causes the unlimited growth of the tumour. Thus tar in some way in the above example excites the first change. It does not, however, change normal cells into sarcoma cells in culture nor does it cause the unlimited growth. (It should be noted, however, that Fischer (19) claims to have transformed by means of arsenious acid, and tar, cultures of the spleen of hen embryos into malignant tumours of the Rous type.) Carrel, however, in this philosophical discussion of the origin of sarcomata does not come to close quarters with the real crux of the matter. He does not attempt to answer the question as to what essentially the Rous agent may be and how it comes to be formed in the first instance.

Carrel and his co-workers have been responsible for the bulk of the work of the type just described. Lewis and Andervont (20) have added to it, however. They found that the malignant cell of the Rous and indol sarcoma is a hypertrophied mononuclear leucocyte which contains the infecting agent. They also found that the injection of blood leucocytes and plasma, from Rous and indol sarcoma fowls without metastases, into other fowls produced the disease in them. In this regard the leucocytes were more potent than the plasma. Fischer (21)(22) has also obtained many of Carrel's results.

As it has been considered to have a bearing on the subject,

reference may be made now to the work of some observers who have found growth promoting substances in tumour and other tissues. As these substances act more as a suitable food-stuff for a variety of cell lineages, it will be seen that their consideration does not assist in the unravelling of the crucial problem as already outlined. Chambers and Scott (23) have found growth promoting substances in tumour tissues. They regard them as fairly stable chemical bodies which are apparently derived from nuclear structures. Drew (24) discusses in detail the nature of the action of embryonal extract. The presence of activating substances in cold extracts of malignant tumours, he suggests, indicates that it is by means of a continuous production of the substances that tumour growth is maintained. He regards the fact, that tumour tissues contain growth stimulating factors while normal tissues require to be severely damaged before they are produced, as a new and very striking difference in behaviour. Burrows (25) thinks that growth in a tissue depends on the crowding of the cells together and a cutting down of their relative blood supplies. Hence there is an accumulation of a substance which stimulates the normal metabolism of the cell. According to Burrows, cancer is the result of any condition or substance which can cause the formation of a dense mass of cells relatively poor in blood vessels within the organism.

Carrel and Baker (26) have themselves made observations on this subject with regard to the chemical nature of some substances required for the growth of fibroblasts and epithelial cells. They found that tissues, cultivated in digested fibrin, showed four times as much growth as their controls in embryonic juice. The synthesis of protoplasm by these cells, when fed on proteoses, leads them to suppose that the marvellous effect of embryonic juice on tissue growth is merely due to a special condition of its proteins which renders possible their splitting into proteoses by the action of the fibroblasts and epithelial cells. Elsewhere (27), Carrel found that the growth of fibroblasts is dependent on the presence in the pericellular fluid of certain substances

which are found in embryonic juices and in leucocytic secretions. These substances differ from hormones because they take part in the synthesis of protoplasm and do not act merely as catalysts of growths. They are called trephones. Epithelial and connective tissue cells do not build up protoplasm from serum. Lymphocytes and large mononuclears can live in serum and manufacture from its constituents growth promoting substances. They are capable of being the nurses or trephocytes of fibroblasts and epithelial cells. It is possible that this is an important observation, it may be remarked, in connection with the coexistence of monocytes and fibroblasts in tumours of the Rous type.

One side of the question of the genesis of tumours has thus been presented, and there would seem to be no escape from the position taken up by Carrel, that tumours such as those of Rous are not caused by a multiplying particulate virus, either directly or indirectly. His further conceptions of the nature of tumour genesis will be discussed later when another view regarding the nature of tumour formation has been dealt with.

In July, 1925, Gye, working with Barnard (28) propounded a theory regarding the causation of cancer. It is not possible to give here full details of the work on which it is based. What may be regarded as the key observations will alone be dealt with.

Gye experimented with Rous sarcoma No. 1, as Carrel had already done. In cultural experiments he added a fragment of Rous sarcoma to a tube containing 5 c.c.s of Hartley's bouillon with 0.2 per cent. KCl and 1 c.c. of fresh rabbit serum. This was incubated aerobically or anaerobically. He found that the culture fluid retained its infectivity in favourable conditions, *e.g.*, large fragment of tissue and strict anaerobiosis, for a week. Gye called this preparation a primary culture. He further ground sarcoma substance with sand and added 5 grammes of the ground material to 100 c.c.s Ringer's fluid. This was filtered through paper pulp and sand. The filtrate obtained was cell free.

Chloroform was then added to the clear filtrate in certain proportions which was then incubated at 37° C. for three and a half hours.

He found that the primary cultures lost their infective action of their own accord, so to speak, in two to seven days, while the filtrate with the above treatment with chloroform lost its infective power also. He observed, however, that the injection of a mixture of a moiety of the two (which moieties separately failed to produce a tumour), now succeeded in causing a growth. (It may be noted in passing that if each of the moieties contained just less than a minimal dose the two when mixed together would supply more than a minimum infecting dose provided one substance only was in question. This possibility is not considered by Gye, but it is emphasised by Simon and Beck (29)).

Gye interpreted these results as meaning that there was present in the primary culture a multiplying particulate virus and a specific chemical factor, the first of which remained alive while the latter disappeared spontaneously, and that in the treated filtrate the chloroform killed the multiplying virus while the chemical factor also present remained presumably unaffected.

These are two basal experiments. Others were performed. Their nature and scope may be indicated by the conclusions arrived at.

Gye thinks that all malignant new growths contain an ultra-microscopic virus—or group of viruses—which can be cultivated. This applies to the carcinomata and sarcomata of mice, fowls, rats, dog and man. The virus resides probably within the cell of the neoplasm. The virus alone, washed free from all adherent material, does not produce a tumour when injected, and does not even produce a visible lesion : but when injected with virus-free extracts of tumours there is produced a malignant new growth. The extracts contain, therefore, a substance called by Gye the specific factor, which enables the virus to attack the cells of the injected animal so as to transform them into cancer cells. There is no species specificity so far as the virus is concerned : for

tumours can be obtained in one species of animal with the virus obtained from the tumour of another species. The specific factor shows a very strict specificity of species. Thus in order to produce a malignant new growth in a mouse it is necessary to use the specific factor from a mouse tumour, while the specific factor of a chicken tumour is ineffective. There is probably also a strict specificity of tissue for the specific factor. So far only sarcomata have been obtained and these only with a mixture of virus and the specific factor from sarcomata, not from carcinomata.

In a later paper in 1926 (30) Gye has reasserted his position. In addition to chloroform, already used as an inactivating agent or destroyer of the particulate virus in his first communication, he has used carbolic acid, mercuric chloride, toluene, hydrogen-peroxide, formalin, acriflavine and hydrocyanic acid. To minimise risk from the variable susceptibility of different fowls, he has used multiple inoculations into single birds. It may be added, however, that while Carrel in his work took great pains to determine the biological and other characters of his single agent and so prepared the way for further experimentation, Gye does not appear to have done so with his two. Consequently in his experimentation we have to deal not with one but with two variable factors, regarding both of which there is less sure knowledge than is the case with Carrel's one.

In discussing Gye's work it needs to be emphasised again at the outset that Carrel's work provides overwhelming evidence for the non-existence of a multiplying particulate virus. In addition, Cori (31), Murphy (32), and Flu (33) have found that cultures of normal tissues can be successfully substituted in Gye's experimental scheme, as already outlined, for the malignant tissue culture which Gye used. This further rules out the possibility of the existence of the multiplying particulate virus: for Gye regards in his experiments such cultures as the source of his particulate virus. Further Mueller (34), Harkins, Schomberg and Kolmer (35), Cori (*loc. cit.*), and Flu (*loc. cit.*)[1] have repeated Gye's

[1] *Vide* also Mueller, Mueller, Marx, Graybill and Sherwood. *Journ. Expt. Med.* 1927, 45, 243.

experiments and all have emphasised the difficulty of duplicating them. Finally Gye, as the basis of his scheme of research, would appear to assume as axiomatic certain occurrences which are at least debatable. He takes for granted that such substances as are mentioned above, if they destroy or diminish the power of tumour production in the filtrate, must do so by acting as an antiseptic on a particulate multiplying virus : with the implication also that these substances have no action soever on the chemical substance or specific factor which Gye supposes to be present in the filtrate. One knows of no warrant for such an assumption, and any positive knowledge on the subject is entirely against its likelihood.

Carrel has illustrated his view of the nature of the action of the Rous agent by comparing it with the bacteriophage. As, however, little is known definitely regarding the innate nature of the bacteriophage, which acts in quite a different sphere, this tells us little regarding the essential character of the Rous agent.

The crucial point in connection with the whole problem is how in the first instance, in primary cases of the disease, the Rous agent appears. If no absolute explanation of this can be found, it may be possible, nevertheless, to identify its origin and action with that of other substances, regarding which knowledge is greater.

In leucotic fowls in has been shown (36) that the primary marrow lesion is brought about not by a single specific cause but is of diverse aetiology. The resultant pathological changes, however, are one in kind, differing only in degree. The secondary foci, tumours, etc., in this disease, are not metastases in the ordinary sense, produced from escaped living tumour cells. They are local manifestations of a disease of a system, the reticulo-endothelial system, secondary to the primary disturbance in the marrow. In the primary focus, owing to the hyperplasia of the myeloid cells caused by the non-specific irritant, there would appear to be produced in excess by the myeloid cells a substance which, circulating in the blood, has induced in the cells of the

reticulo-endothelial system, in distant parts of the body, the formation of cells of the same type as in the original focus. This substance, without prejudging, may be called a stimulin. Its specificity is of a double nature in that it not only causes cells of a specific type to be formed but these specific cells, when formed, again produce the specific stimulin.

This stimulin is lineage specific in that it stimulates the production of cells of the myeloid lineage only. It does not for instance stimulate the monocytic lineage. It would appear also to be individual specific in that cell-free extracts of the lesions (containing the stimulin) injected into fowls of the same species, breed, or even blood relation, do not produce the lesions in their reticulo-endothelial system. (In making this statement, Ellermann's claims in this direction have not been lost sight of—*vide* McGowan, pp. 19, 20.)

The same description virtually holds for the occurrences in tumours of the Rous type with certain emendations. In this case the lineage specificity is not so rigid, corresponding in fact with the greater developmental potentialities of the free-histiocyte or monocyte. Thus, in fowls with Rous tumours, there is myeloid lineage implication in addition to the free-histiocyte one. The individual specificity also is not so rigid. In first cases, the specific stimulin does act on the reticulo-endothelial system of other fowls. This, however, may be regarded as being only an extension of the reticulo-endothelial system of the primarily affected fowl, for blood-relations only are susceptible. By prolonged and repeated transmissions, the tumour may be acclimatised to the reticulo-endothelial system of the breed or even of the species.

The condition as here outlined would appear to have its roots deep down in embryological happenings and would seem to be a perversion of a normal embryological process, whereby, by means of cell secretions, the various parts of the reticulo-endothelial system in the embryo is parcelled out harmoniously into various functioning tissues and organs. In both the instances described, cells with great residual developmental potentialities have been affected and the cell

lineage—the monocytic one—with the greater potentiality has exhibited the greater capacity for systemic spread in the individual and in the breed or species. The question arises whether other sarcomata, of the ordinary type, may not have a similar, if more restricted, systemic spread. Jolly (37) has indicated it as his belief that this is possible. Conversely, cells which have reached their developmental apogee,[1] such as epithelial tissues, show no systemic spread. Their metastases are true ones, arising from transplanted cells: and they cannot be transmitted to other animals by cell-free filtrates.

In leucosis, as already indicated, the originating cause is of diverse nature. Rous tumours have been originated by the action of such non-specific substances as tar, indol, and arsenic. In the sporadic tumour cases of Rous type in this series, the primary tumour was either subcutaneous—and so liable to have been caused by traumatism—as in the case of the weasel bite, or in an organ, which could easily be affected by infectious or inflammatory conditions, thus the kidney and especially the left one in the fowl and the lung. The ovary was also the seat of origin of the disease fairly frequently. This is probably due to the ovary being for long periods in a condition of physiological inflammation owing to the intense anabolism and katabolism. In one case—cock 91—a sarcoma of the Rous type, of the size of a Jaffa orange, appeared, following on the inoculation of sterile powdered glass into the peritoneal cavity. Its pedicle of attachment to the covering of the duodenum was no thicker than a lead pencil and was situated at a part of the abdomen liable to have been injured by the point of the needle on inoculation as it was performed. In this case for special reasons the inoculation had been made in the right hypogastrium. In addition, to allow of the passage of the powdered glass, a large needle, made as a matter of fact from a small metal catheter, was used. This case is of interest in connection with the fact that Rous, Gye, and

[1] Such cells are derived from division of similar cells. The others are continuously arising from basal stem or primitive mesenchyme cells.

others have found it of advantage to inoculate a small quantity of silica along with their filtrates of Rous tumour. Of course no Rous agent was injected in the present instance.

It is a matter, possibly, for consideration whether the agents of some other diseases caused by filtrable viruses may not be in some cases of the nature of the stimulin found here. In such cases, the increase in quantity of the virus would not be due to a direct multiplication but would be caused indirectly by its effect on living tissue cells. These would be caused to multiply and in so doing would produce more of the chemical agent, a phenomenon which would usually be interpreted as a multiplication of a living particulate virus.

The origination of tumours of myeloid and free-histiocytic tissues by non-specific irritants in the fowl has been discussed. The subject would be incomplete without a reference to tumours of the lymphatic lineage, especially as one has not met with as yet a tumour of this type in the fowl. It is difficult at present to assign a reason for this. The only pertinent point one knows of meantime is that lymphatic glands and some other lymphatic tissues are not found in the fowl. Ewing (38), in his introduction to the sections on lymphomas and lymphosarcomas, remarks as follows: 'Two important physiological properties influence the conception of tumours of the lymphoid tissue: (1) lymphoid tissue responds to irritation with inflammatory hyperplasia far more actively than any other tissue. (2) lymphoid tissues are relatively mobile rather than fixed. This renders difficult the distinction between simple and neoplastic hyperplasis of lymphoid tissue.'

A third characteristic of lymphatic tissues, which has been enlarged on somewhat in a previous chapter, is that they stand across the path of foreign material attempting an entrance into the blood. This sieve action is especially exhibited by the lymphatic glands, with the additional factor that, once they have intercepted the material, they cannot get rid of it as readily as some other lymphatic

tissues. Consequently it remains to cause irritation. Perhaps in this circumstance may be found the explanation of the absence of lymphatic tumours in fowls.

SUMMARY

Attention is directed to the fact that the crucial problem in connection with tumour formation of the fowl is the elucidation of the origin of the agent in primary cases.

Sarcomatous tumours of fowls, including those of a leucotic nature, are caused, in all probability, by irritation from non-specific irritants of various types and origins.

This irritation causes hyperplasia of various cell lineages. The hyperplasia is associated with the over-production of a specific cell stimulin. This stimulin produced by one type of cell causes in its turn the origination, from undeveloped cells, of the same type of cell which produced it. In normal conditions, the various cell lineages, developed from the reticulo-endothelial system, would appear to be kept in harmonious relation to one another and to the whole body by there being an adequate and balanced control of the corresponding stimulins. In tumour cases, however, control would appear to be lost and one stimulin, produced in excess, runs riot and causes unrestrained growth, in reticulo-endothelial areas throughout the body, of its corresponding cell.

The specificity of the stimulin is at times so rigid that growth may be confined to one cell lineage of the reticulo-endothelial system of an individual fowl. Sometimes, as in the case of Rous tumours, it may affect more than one lineage owing to the pluri-potential nature of the elementary cell involved. A relaxation of rigidity may also occur in regard to the animal, considered as a whole, in which the stimulin may produce its effect. In such cases, the order of progress is from the reticulo-endothelial system of the original fowl, through that of blood relations, to that of the breed as a whole and finally to that of the species.

The occurrence and activity of the stimulin is associated with the developmental potentialities still remaining in the

cell. It is thus greater in cells of the order of the monocyte or free-histiocyte than in those of the myelocyte or myelocytic histioblast. It would appear to be absent in cells of the epithelial type which are completely differentiated. Corresponding with the latter, is the fact that, in epithelial cancers, there is not systemic spread, and transference to other parts of the body and other animals is by means of living tumour cells.

The specific stimulin would therefore appear to be an integral part of the bionomics of normal undifferentiated cell lineages, which, by becoming uncontrolled, causes the production of tumours. As regards the transmission of the disease by the stimulin, it seems evident that the inoculation of just sufficient of it, say in the case of the Rous sarcoma, to affect a single monocyte would be adequate, other conditions being favourable, to determine the formation of a tumour. An ever-increasing vicious circle would be set in this way ; stimulin being produced thereby in larger quantity to affect a larger number of tissue cells with a further greater increase in the amount of stimulin. Perhaps this conception lies at the root of the mode of action of some other so-called filtrable viruses.

IX

SUMMARY AND CONCLUSIONS

As a preliminary step to facilitate subsequent discussion, a scheme of blood cell origin and formation, based largely on previous observations on blood diseases in fowls, has been outlined in Chapter I.

Observations have been made on fowls, the bearers of experimentally produced Rous sarcoma No. 1, as regards general effects and reaction to trypan blue intravital staining. They have also been carried out on fowls, the subject of naturally occurring tumours of the Rous type.

Blood examinations showed a quite definite monocytosis. Histological examination of the tumours themselves showed that the chief cell affected was the monocyte or free-histiocyte. As this cell cannot of itself form a tissue, this is accomplished by its being transformed into a fibroblast. Cell multiplication in the tumours took place by mitosis in the enlarged and dropsical monocytic cells. It also occurred, however, by amitotic division in the precursors of the monocytic cells, the histioblasts. No evidence of multiplication was seen in the fibroblastic cells.

The tumour is a local manifestation of disease in a system, the reticulo-endothelial system. The cells affected in this case are the monocytes or free-histiocytes. The spread of the disease away from the body of the tumour takes place by proliferation in the perivascular region of arteries. This locality is a great storehouse for reticulo-endothelial elements. When the disease has progressed somewhat, other lineages of the reticulo-endothelial system are affected secondarily, giving rise to leucotic pictures.

The spontaneous tumours met with in fowls were of the Rous type and showed all gradations between very cellular fibroblastic tumours and dense fibromas. In only one of these tumours were transplanting experiments attempted. The tumour developed in the first grafting, but was lost in the second. The same thing happened with the Rous No. 1 sarcoma. In the first two fowls, white leghorns, the tumour grew vigorously; while in the next transplanting, again into white leghorns, the tumour in many cases did not grow. Tumours of this type seem to be of fairly frequent occurrence in fowls and resemble the Rous No. 1 very closely histologically. Transplantability into other fowls, as a criterion for resemblance, for reasons inherent in the nature of the tumour should not be insisted on too closely.

These spontaneous tumours were associated secondarily with the presence of leucotic lesions of a myeloid type.

Evidence was adduced from their mode of occurrence that irritants of a nonspecific type may play a part in the origination of the tumour process.

In a series of fowls, leucotic tumours of various types shading off into ordinary leucosis were observed and their derivation from various cells in the myeloid lineage pointed out. These conditions are again diseases of a system, the reticulo-endothelial system. In contradistinction to Rous tumours which were associated with secondary leucotic lesions, these changes were not associated with secondary Rous changes.

The question of the essential malignancy of these conditions was discussed and reasons were adduced for considering the malignant types to be malignant growths of enhanced malignancy.

In considering the various problems raised by such transmissible tumours, it is emphasised that the crucial point in connection with tumour formation in the fowl is the elucidation of the origin of the provocative agent in primary cases of the disease.

In a previous work, it was shown that leucotic conditions are brought about in the fowl by diverse causes. Here, in

the same way, it is shown that sarcomatous tumours of the Rous type are conditioned by nonspecific irritants of various types and origins.

The irritation produces hyperplasias of various cell lineages. Such are associated with the over-production of a specific cell stimulin, which causes the continued growth subsequent to the appearance of the primary one. The stimulin, manufactured by one type of cell, causes in its turn the origination, from undeveloped cells of the reticulo-endothelial system, of the same type of cell which produced it. In normal conditions the various cell lineages arising from the reticulo-endothelial system appear to be kept in harmonious relation to one another and the whole body by an adequate and balanced control of the corresponding stimulins. In tumour cases, however, owing to the great hyperplasia of a specific cell, proportionate growth is lost and one stimulin, produced in excess, runs riot and causes unrestrained growth of the corresponding cell in reticulo-endothelial areas throughout the body, where the environment favours the growth of such cells.

The specificity of the stimulin is, at times, so rigid that growth may be confined to one cell lineage of the reticulo-endothelial system of an individual fowl. Sometimes, as in the case of Rous tumours, it may affect more than one lineage owing to the pluripotential nature of the undeveloped cell involved. A relaxation in regard to the rigid specificity may also occur in reference to the animal, considered as a whole, in the reticulo-endothelial system of which the stimulin may produce its effect. In such cases, the order of progress is from the reticulo-endothelial of the originating fowl, through that of blood relations, to that of the breed as a whole and finally to that of the species.

It will be seen thus how, unless the very greatest precautions are taken, and even with them, grafts from primary tumours into other fowls may fail to take.

The occurrence and activity of the stimulin is associated with developmental potentialities still remaining latent in the cell. It is thus greater in cells of the order of monocytes

or free-histiocytes from which the Rous tumour develops than in those of the myelocyte or myelocytic histioblast, from which leucotic conditions arise. It is apparently absent in cells of the epithelial type, because of their complete differentiation. Corresponding with this is the fact that, in epithelial cancers, there is no systemic spread, and transference to other parts of the body and to other animals is by means of living cells.

The specific stimulin would therefore appear to be an integral part of the bionomics of undifferentiated cell lineages. If the stimulin belonging to one lineage gets out of hand then tumour production of cells of that lineage occurs. As regards the transmission of the disease by the stimulin, it seems evident that the inoculation of just sufficient of it, say in the case of the Rous sarcoma to affect a single monocyte, would be adequate, other conditions being favourable, to determine the formation of a tumour. There would be thus an increase of the specific stimulin in the specific cell, which would cause other specific cells to be originated with again an increase of the specific stimulin. An ever-widening vicious circle would thus be set up. Such considerations throw light on the extremely small dose of the agent which may be necessary to transmit the tumour and may have a bearing on an understanding of the essential process at work in the case of some other so-called filtrable viruses.

After these remarks it may be more easily understood how the leucoses in their malignant form can be regarded as being not only malignant diseases, but malignant diseases of enhanced malignancy. They have the means not only of spread possessed by ordinary malignant diseases but in addition can spread as part of a system by means of substances, the purpose of which is to cause growth within that system. In this sense, such diseases, within limits, may be regarded as being of the reticulo-endothelial system not of an individual but of the race. In addition, the question may be raised whether all sarcomata have not in them in varying degree, as in an essential part of their being, an element of this system spread.

Melanotic sarcomata and a melanotic carcinoma of the ovary of the fowl are described and discussed. As these occur without any merging of the one into the other, this would seem to show that both types of tumour—the sarcomatous and carcinomatous melanoma can and do exist.

The problem of the origin of the pigment in these cases led to a discussion of many points in relation to the anatomy and physiology of the reproductive organs as well as of the suprarenal gland and sympathetic system. It is suggested that the interrenal body or suprarenal cortex through the medium of the sympathetic, especially as represented by the suprarenal medulla, controls the soma of the animal in many respects, including that of colour, and causes it to conform to the type characters of the race as represented by the germinal epithelium. The suprarenal cortex also may act as a station whereby the alterations of the soma, conditioned by the gonad, are put into effect.

In regard to melanin, it is suggested that the melanogen, which gives rise to the melanin in these cases of ovarian tumour, is derived from a melanogenic lipo-protein complex secreted into the egg yolk as a provision for the colouring of the young chick. Considerations, discussed in the text, raise the question as to whether melanin metabolism may not be centred round the cell which takes the dye in intravital staining—the ' pyrrhol cell ' or macrophage.

No certain source of origin of the melanogenic lipo-protein complex can be suggested except to indicate that it is possibly formed in the organ which is usually regarded as having specially to do with the elaboration of fat products, namely the liver.

A parallelism, in regard to excretion etc., of melanin from the body, is suggested in the excretion of trypan blue and haemosiderin.

Owing to the difficulty of distinguishing the various lymphoid cells as they occur in tumours and to the apparent non-existence of lymphatic tumours in the fowl, some attention has been directed to a discussion of lymphatic tissues in general. The bearing of various points on the

occurrence and development of mesenchyme derived tumours in the fowl, such as Rous tumours, leucotic tumours, and leucosis, is dealt with and fresh interpretations of various anatomical or physiological phenomena met with are submitted.

The essentially similar structure, *qua* haematological tissues, of the liver, spleen, and lymphatic glands is pointed out. In doing so, attention is drawn to the circumstance that endothelial and subendothelial position in *veins* is associated with the formation of myeloid cells, such as red blood cells and myelocytes ; while perivascular position in relation to *arteries* is related to the appearance of lymphocytic cells. This emphasises the histiod nature of lymphatic cells and would appear to indicate a closer relationship, than is usually accorded them, to the monocytic lineage. Free-histiocytes or monocytes may arise in both situations, perhaps justifying a subdivision of them in two types as has already been attempted by Sabin by means of supravital staining.

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PRINTED IN GREAT BRITAIN
BY ROBERT MACLEHOSE AND CO. LTD.
THE UNIVERSITY PRESS, GLASGOW